PTERIDINE PATTERNS DURING DEVELOPMENT AND AGING IN *DROSOPHILA REPLETA*

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PTERIDINE PATTERNS DURING DEVELOPMENT AND AGING IN DROSOPHILA REPLETA

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SUMMARY

Qualitative and quantitative changes in pteridines and purine-based UV-absorbing compounds were analyzed during development and aging in wild type Drosophila repleta from Atlanta, Georgia; Oahu, Hawaii; Yucatan Peninsula, Mexico; Palmar, Costa Rica; and Sydney, Australia by two-dimensional ascending paper chromatography in a modified thin-layer system. Developmental stages were analyzed daily. Adult heads and bodies were analyzed separately on the day of eclosion and at 5, 10, and 15 days after eclosion. Isoxanthopterin concentration in adult bodies was quantitated fluorimetrically at each imaginal age sampled. In conjunction with biochemical studies, development was timed, morphology was compared, and cross-fertility was examined.

Atlanta, Hawaii, and Yucatan differed little either qualitatively or quantitatively in pteridines and UV-absorbing compounds, and differences in isoxanthopterin concentration were not significant. Isoxanthopterin concentration was significantly greater in Costa Rica than in Atlanta, Hawaii, or Yucatan and was significantly greater in Australia than in any other stock. Quantitative visual estimates indicated similarly elevated levels of other pteridines in Costa Rican and Australian stocks. Quantitative sexual differences in pteridines were not detectable at eclosion but were marked in all stocks five days later. Isoxanthopterin concentration increased significantly during aging in males and decreased significantly in females. Visual estimates indicated

similar changes in other pteridines during aging. Atlanta, Hawaii, and Yucatan are similar in morphology and developmental timing and interbreed freely with each other and a recently isolated Atlanta stock. Costa Rica and Australia complete the life cycle 24-48 hours earlier than other stocks, are reproductively isolated from each other and all other stocks, and differ in eye and body pigmentation from other stocks and the classic species description. Results indicate that geographical isolation of Australian and Costa Rican D. repleta has been accompanied by genetic isolation, reflected in reproductive isolation, differences in developmental timing, and both morphological and biochemical variation.

CHAPTER I

INTRODUCTION AND LITERATURE SURVEY

Introduction

During the past seventy years, taxonomy and species morphology in the genus Drosophila and the genetics, development, and biochemistry of wild type and mutant strains of D. melanogaster have been studied extensively. Unlike D. melanogaster and several other species within the genus, D. repleta has not been subjected to extensive genetic, developmental, or biochemical investigation. Only three spontaneous mutants of D. repleta have been described: (1) a scarlet-eyed mutant, which was shown to be a simple, autosomal Mendelian recessive (Hyde, 1915); (2) a sex-linked recessive in which the dorsum of the thorax is lighter in color and has less coalescence of markings than in the wild type (Sturtevant, 1915); and (3) an autosomal dominant mutant for yellow body color (King, 1927). A detailed cytological analysis of the polytene chromosome banding of D. repleta (Wharton, 1943) has not been supplemented by identification and mapping of genetic markers.

There have been two studies examining genetic isolation among stocks of D. repleta collected in geographically different locations (Wharton, 1941, 1942; Humphrey, 1974; Colley and Humphrey, Manuscript submitted for publication). Celeste M. Humphrey (1974) investigated genetic isolation among six stocks of D. repleta, five of which are included in the present study. Geographical isolation of stocks from

Australia and Costa Rica appeared to have been accompanied by reproductive isolation, while stocks from Georgia (Atlanta), Connecticut, Mexico (Yucatan), and the Hawaiian island of Oahu formed a freely interbreeding group.

Several morphological differences, including differences in pigmentation of eyes and bodies, also phenotypically distinguish the Australian and Costa Rican stocks from each other and from the group consisting of the other four stocks. For example, the mature adult D. repleta is classically described as having sepia eyes and dark brown bands on the dorsal surface of the abdomen (Patterson, 1943). The Atlanta, Connecticut, Yucatan, and Hawaii stocks conform to the classic description of the species in pigmentation, as well as in reproductive morphology, while Australian and Costa Rican stocks differ in morphology of spermathecae and ventral receptacles and in pigmentation. The eyes of mature Australian flies are brick-red, and the testes are much brighter yellow than in any other stock. The Costa Rican stock has bright red eyes which do not darken with age, and the dorsal abdominal bands are paler brown, producing the effect of a distinctly lighter body color than for other stocks.

These phenotypic manifestations of differences in genetic constitution among stocks initially suggested investigation of the extent to which differences in pigmentary phenotype might be attributable to differences in pteridine patterns, rather than to differences in quantities of melanin or ommochromes. Colley (1967) analyzed pteridine patterns of the Atlanta stock during development and aging, and

indications of qualitative and quantitative variations in pteridine patterns of different stocks and interstock hybrids were found in preliminary analyses in the present study.

In view of the limited information on duration of stages in the life cycle of D. repleta (Frappa, 1931; Colley, 1967; Colley, personal communication), the absence in the literature of a daily sequential developmental analysis of pteridine patterns in this species, and the limited information concerning either developmental or adult pteridine patterns in D. repleta (Hubby and Throckmorton, 1960; Throckmorton, 1962; Colley, 1967; Howell, 1969), the present characterization and comparative biochemical analysis was undertaken. Changes in patterns of pteridines and UV-absorbing compounds during development and aging in five stocks of D. repleta from different geographical regions were analyzed by two-dimensional ascending paper chromatography, followed by visual quantification of all compounds isolated at each sampling interval and fluorimetric determination of isoxanthopterin concentration during aging of adults.

Three primary areas of investigation were of major interest. First, we were interested in determining the distribution and timing of appearance and/or disappearance of pteridines and pteridine-related compounds among the five stocks under consideration. Secondly, the characterization of pteridine patterns during development and during maturation and aging of adults could provide additional evidence elucidating pathways proposed for pteridine metabolism in the genus Drosophila (see Literature Survey). Thirdly, since the timing of appearance of intermediates and products of enzymatically catalyzed reactions reflects timing

of regulation of enzyme synthesis and/or activity, it ultimately reflects the genetic constitution of the organism. Thus, a comparative biochemical study of this type should provide information concerning interstock genotypic similarities and differences, with particular reference to intraspecific genetic variation in the pathways of metabolism of pteridines and related purine derivatives.

Literature Survey: Pteridines in Drosophila and Other Organisms

Hopkins (1889, 1891, 1895) and von Urech (1894) reported results of investigations into the wing pigments of butterflies. Hopkins (1889) reported the isolation of a white pigment which he believed to be closely related to uric acid. Neither von Urech nor Hopkins recognized the butterfly wing pigments as members of a then unidentified group of naturally occurring compounds. Wieland and Schopf (1925, 1926) identified these compounds as members of a group which they designated the "pterines," from the Greek for "wing" (Wieland and Schopf, 1925). The first pterine pigments isolated in pure form were named according to their color and source: xanthopterin ("yellow wing") and leucopterin ("white wing"). The term "pteridine" was introduced by Schopf and Becker (1936) to identify those compounds responsible for wing pigmentation in the family Pieridae. The terms "pterine" and "pteridine" were used interchangeably by early researchers to distinguish these pigments from other groups of compounds which contribute to the total pigmentary phenotype.

Attempts at structural determination of pteridines encountered experimental difficulties. Purrmann (1940, 1941) unequivocally demonstrated the chemical structure of xanthopterin, leucopterin, and

isoxanthopterin. These three pigments have a bicyclic nitrogenous ring system, systematically termed a pyrimido-(4,5-b)-pyrazine. The term "pteridine" is used as the common designation of this structure. The system for numbering these compounds is that employed by Kuhn and Cook (1937) for aza-derivatives of naphthalene. Pfleiderer (1964) suggested that, since the naturally occurring pteridines have trivial names ending in "pterin" and since a majority of them are derivatives of 2-amino-4-hydroxypteridine, the term "pterine" should be restricted to the 2-amino-4-hydroxy derivatives.

Phenotypic variation in the pigmentation of eyes and bodies of Drosophila and other insects has been recognized and studied since the early 1900's. Genetic and biochemical studies of phenotypic variation in eye color in Drosophila were initiated in the mid-1930's (Beadle and Ephrussi, 1937; Beadle and Thimann, 1937; Ephrussi and Chevais, 1937). Genetic aspects of these and other studies have been reviewed by Ziegler (1961).

Mainx (1938) demonstrated that two major groups of non-melanin pigment contribute to Drosophila eye color, later identified as ommochromes and pteridines. He demonstrated differences between the two pigment groups in water solubility and in fluorescence after reaction with hydrogen peroxide. The ommochromes, brown water-insoluble pigments, are deposited 53-55 hours after the onset of pupation in D. melanogaster (Danneel, 1941). Danneel's report that the red, water-soluble pigment component, which comprises the pteridines, appeared approximately 71 hours after the onset of pupation was based on the time at which a red water-

soluble pigment could be extracted. His extraction methods did not provide for recognition of the appearance of pteridines other than the drosopterins, which appear red in visible light. Other pteridines certainly are present at earlier developmental stages (Hadorn and Mitchell, 1951). The ommochromes are present as eye pigments throughout the phylum Arthropoda, and a number of pteridines are widely distributed in the Insecta. Drosopterin, isodrosopterin, and neodrosopterin are unique to the genus Drosophila (Viscontini et al, 1955), with two exceptions (Throckmorton, 1962). Maas (1948) demonstrated that the red eye pigment component of Drosophila could be resolved by chromatography into at least eight different compounds. In the same year, Williams and Kirby (1948) demonstrated the applicability of ascending chromatography to separation of complex mixtures of compounds.

Hadorn and Mitchell (1951) reported results of an ascending paper chromatographic study of developmental changes in patterns of fluorescing compounds in D. melanogaster and of mutant genotypes which altered the wild type coloration of eyes or bodies. They reported the presence of at least seven fluorescent compounds and two ninhydrin-positive peptides in wild type D. melanogaster and found striking changes in the concentrations of fluorescent substances during development. Mutant adults (3-4 days after eclosion) differed from wild type both in total number of fluorescent compounds and in quantities of individual compounds. Sexual differences in quantity and tissue localization of pigment during development were observed in wild type D. melanogaster. Based on results of their study, Hadorn and Mitchell concluded that several of the fluorescent compounds present were identical or related to the "red eye pigment

component" described by earlier workers.

Buzzati-Traverso (1953) examined qualitative differences in patterns of fluorescent compounds in bodies of ten-day-old adults (1) of morphologically identical stocks of D. melanogaster of different geographical origin, (2) of a limited number of phenotypic mutants of D. melanogaster which did not affect either eye or body color, and (3) of heterozygotes in which genotypic differences were masked by dominance. Buzzati-Traverso verified the report of Hadorn and Mitchell (1951) that diet and environment did not affect the pattern of fluorescent compounds for any particular genotype and observed (1) that stocks of the same species from different geographical regions had similar but distinct patterns (differing either in total number of fluorescent spots or in quantity of material present); (2) that aging of adults has little effect on the male pigment pattern but does alter quantities of a number of compounds in females until approximately the tenth day after eclosion; (3) that phenotypes other than those affecting eye or body color may exhibit differences in patterns of fluorescent compounds; and (4) that in some stocks studied at least nine fluorescent compounds are present.

Paper chromatographic techniques have been used to study evolutionary and taxonomic problems in Drosophila, to demonstrate biochemical differences and similarities in comparative studies of mutants and different species, and to study interspecific differences in patterns of accumulation of fluorescent compounds. Numerous investigations of pteridines in Drosophila have been directed toward or have provided information leading to identification of newly isolated fluorescent compounds as

pteridines and elucidation of metabolic relationships among the compounds isolated (Forrest and Mitchell, 1954a,b,c, 1955; Viscontini et al, 1955, 1959a,b; Forrest, Glassman, and Mitchell, 1956; Hadorn, 1956, 1959; Forrest, Hatfield, and van Baalen, 1959; Hubby and Forrest, 1960; Hubby and Throckmorton, 1960; Taira, 1960; Throckmorton, 1962; Harmsen, 1966b; Colley, 1967; Howell, 1969; Rembold, 1970). Pteridines which appear to be related to the drosopterins and to each other through oxidation-reduction steps include (1) biopterin, which is colorless in visible light and has a blue fluorescence (Forrest and Mitchell, 1955; Nathan and Cowperthwaite, 1955); (2) sepiapterin and isosepiapterin, which are yellow in visible light and yellow-fluorescing (Hadorn and Mitchell, 1951; Forrest and Nawa, 1962, 1964); and (3) tetrahydrobiopterin (Ziegler, 1960, 1963). Pteridines which are not in the direct pathway of drosopterin synthesis but are related as pterine catabolites in Drosophila include (1) xanthopterin (light yellow in visible light, green fluorescence); (2) isoxanthopterin (colorless in visible light, blue-violet fluorescence); (3) 2-amino-4-hydroxypteridine (pterine, AHP), which is colorless in visible light and has a blue fluorescence; and (4) pterine-6-carboxylic acid (colorless in visible light, blue fluorescence).

A simplified structural representation of the 2-amino-4-hydroxypteridines, or pterines, is shown in Figure 1 (modified from Rembold and Buschmann, 1964; Gilmour, 1965; Ziegler and Harmsen, 1969). While the structures of the pterines are represented in much of the literature as 4-hydroxypteridines and are designated as such in a majority of the biochemical literature, recent considerations from an energy point of view

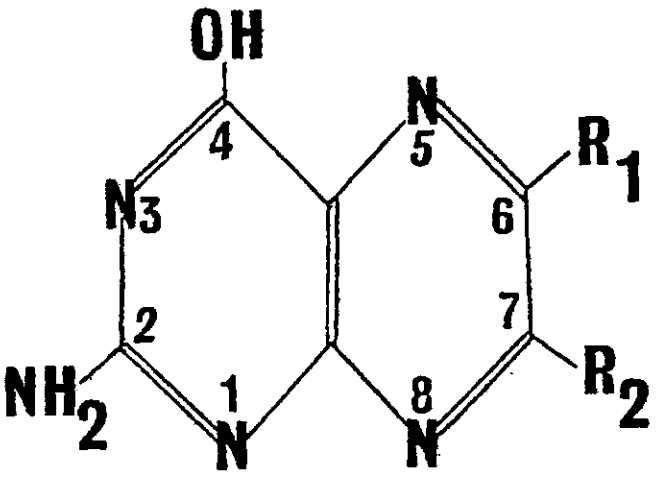

Figure 1. Simplified Structural Representation of Pterines (2-Amino-4-Hydroxypteridines). (Modified from Rembold and Buschmann, 1964; Gilmour, 1965; Ziegler and Harmsen, 1969.) Pterines may have a simple group substitution (e.g., methyl, carboxyl, hydroxyl) at carbon 6 and/or carbon 7 or a polyhydroxy side chain group substitution at carbon 6 or carbon 7. The 7,8-dihydrogenated and 5,6,7,8-tetrahydrogenated pterines may or may not have group substitutions.

indicate that the majority of these compounds exist in equilibrium in aqueous solution in the tautomeric 4-oxo form rather than the 4-hydroxy form. For similar energy considerations the 6- and 7-hydroxy structures are considered to exist mainly as 6- and 7-oxo derivatives (Brown and Mason, 1956; Pfleiderer et al, 1960; Brown and Jacobsen, 1961). The use of the 2-amino-4-hydroxy term follows the trivial usage which persists in the literature.

The pteridines have been subdivided further into conjugated and unconjugated pteridines. The naturally occurring conjugated pteridines include folic acid and its derivatives. The derivatives comprise compounds with a p-aminobenzoylglutamic acid residue attached to the 6-methyl group of 2-amino-4-hydroxy-6-methylpterine (6-methylpterine).

There are four major subgroups of unconjugated pteridines: (1) pterines which have a simple group substitution at carbon 6 and/or carbon 7 (e.g., a methyl, carboxyl, or hydroxyl group); (2) pterines having a polyhydroxy side chain group substitution at carbon 6; (3) pterines with a polyhydroxy side chain group substitution at carbon 7; and (4) the hydrogenated pterines, which may or may not have group substitutions. The hydrogenated pterines may be further subdivided into 7,8-dihydrogenated and 5,6,7,8-tetrahydrogenated pterines.

The chemical, physical, and biological characteristics of the pteridines have been described and discussed extensively in reviews (Mason, 1954; Albert, 1954; Ziegler, 1961; Pfleiderer, 1963; Ziegler and Harmsen, 1969; Blakley, 1969; Rembold and Gyure, 1972) and by participants at the International Symposia on Pteridines (1954, 1964, 1970).

The reader is referred to these reviews and to Proceedings of the Symposia for a more comprehensive treatment of pteridine biochemistry.

Briefly, properties of pteridines having metabolic significance of interest to the present study include the following: (1) The presence of the 2-amino group and the 4-oxo group of the pterines makes them amphoteric in nature. (2) The simple pterines are somewhat soluble at both high and low pH values but tend to autochelate at neutrality and exhibit low solubility in organic solvents. (3) The complex substituted and conjugated pteridines are water-soluble but are only slightly soluble in organic solvents. (4) The anions and cations of the pteridines are all considerably more soluble in aqueous solutions. (5) The pteridines are chemically and biologically associated through oxidation-reduction steps, common precursors or intermediates, and common enzyme systems. (6) Pteridines free of association with protein carriers fluoresce when irradiated with ultraviolet light of high wavelength. Their UV spectra generally exhibit a maximum wavelength greater than 300 nm, which helps to distinguish them from purines and pyrimidines. The colorless pteridines absorb strongly between 340 and 380 nm, while the absorption spectrum of colored pteridines may extend into the visible range, up to approximately 500 nm. (7) The stability of the pteridines is increased by the presence of electron-liberating substituents (e.g., $-NH_2$ or -OH), except in the cases of the reduced derivatives. (8) The stability of the pteridines under both physiological and non-physiological conditions is, in decreasing order of stability, simple substituted > polyhydroxy C-6 or C-7 substituted > dihydropteridines > tetrahydropteridines.

Drosophila melanogaster has been shown to be capable of the biosynthesis of folic acid (Sang, 1956), but it requires supplementary folic acid in the diet (Sang, 1962). Among the larval growth requirements of D. melanogaster is riboflavin (Tatum, 1939). While some authors (Nathan et al, 1956; Blair, 1961) have suggested that, in larvae and non-pteridine-accumulating adults of certain insect species, all pteridines present could be accounted for as end products arising from catabolism of dietary folic acid and/or riboflavin, the possibility of de novo synthesis of pteridines by these organisms from basic components of the general metabolic pool (Harmsen, 1966a) cannot be eliminated. Studies of mammals deficient in both dietary folic acid and riboflavin revealed that these animals excreted biopterin in normal amounts (Pabst and Rembold, 1966; Fleming and Broquist, 1967). Ziegler and Harmsen (1969) reported a personal communication from R. R. Jones (1968) that Pieris raised on a chemically defined diet with low folic acid content still had normal levels of pteridines which could only be explained in terms of biosynthesis from a non-pteridine-containing precursor.

Prior to 1959, studies of the synthetic pathways of pterines and the pteridine ring had been formulated along lines of investigation recalling the early work of Hopkins (1889). Hopkins in fact suggested that the pigment he had isolated was a derivative of uric acid utilized as a "dry storage" product for disposal of nitrogenous waste; the pigment was demonstrated to be even more closely related to purines than he had suspected. Following the elucidation of the structures of a number of the naturally occurring pterines and the pteridine ring system (Purrmann, 1940,

1941), the work previously reported by Becker (1937) was interpreted as indicating the presence of a labile, non-fluorescing hydrogenated precursor. Due to the structural resemblance of guanine and guanosine to the pterines and other pteridines, a biological precursor-product relationship had been suspected for some time (e.g., Hadorn and Mitchell, 1951). Nathan and Cowperthwaite (1955) demonstrated that a fluorescent compound present in some commercially available samples of guanosine was, in fact, biopterin. Albert (1957) demonstrated the feasibility of a chemical conversion of purines to pteridines and postulated that the reaction proceeded by a series of steps involving the opening of the imidazole ring of the purine, with subsequent loss of carbon 8.

The first definitive *in vivo* and *in vitro* studies of biosynthetic pathways of the pteridine ring and the naturally occurring pteridines began in 1955 with the work of Weygand and Waldschmidt, who injected ^{14}C-labeled precursors of the purines into the pupae of butterflies to investigate the hypothesis that naturally occurring pteridines were biosynthetically derived from purines. Investigations following this initial work expanded the use of labeled precursors of purines and the use of labeled purines, as well as the use of labeled pteridines, and extended the studies to include other insects, bacteria, bacterial extracts, and isolated insect organs and tissues.

Weygand and coworkers (Weygand and Waldschmidt, 1955; Weygand et al, 1961; Simon et al, 1963) studied biosynthesis of leucopterin in the butterfly species *Pieris brassicae* and *Gonepterix rhamni*, making use of glycine-1-^{14}C, formate-^{14}C, and 4-amino-4-imidazolecarboxamine-4-^{14}C. These

precursors of purines were found to label the pteridines to a similar extent. Degradation studies showed that the pattern of labeling of leucopterin corresponded to the position of the label in the purines. Brenner-Holzach and Leuthardt (1959, 1961) fed labeled glucose to larvae of D. melanogaster and found that the isolated pteridines were labeled. Initial studies with glucose-1-^{14}C and glucose-U-^{14}C provided evidence that carbon atoms 6 and 7 of the pyrazine ring were derived from a sugar precursor (ribose or glucose). Later studies (Brenner-Holzach and Leuthardt, 1967) showed that administration of glucose-6-^{14}C produced marked labeling of biopterin and drosopterins in the 3'-position of the side chain, whereas glucose-2-^{14}C led to marked labeling at the 2'-position. Results of these studies led to the hypothesis that glucose incorporation into pteridines of Drosophila occurs via conversion to ribose, with incorporation of ribose into a purine nucleotide which finally is incorporated into the pteridine structure and side chain at C-6.

Work of a number of authors with bacteria and bacterial extracts has indicated that neither adenine-8-^{14}C nor guanosine-8-^{14}C is incorporated into the initial pteridine ring (Shiota and Disrealy, 1961; Vieira and Shaw, 1961; Reynolds and Brown, 1962, 1964; Baugh and Shaw, 1963; Krumdieck et al, 1964, 1966; Shiota et al, 1964, 1967, 1970; Shiota and Palumbo, 1965; Burg and Brown, 1968; Brown, 1970). These workers have also demonstrated that the loss of carbon 8 is due to its splitting off as formic acid.

Watt (1967) demonstrated that generally labeled guanosine was incorporated into a number of the naturally occurring pteridines of pupae or excised developing wings of the butterfly Colias eurytheme to a greater extent than was generally labeled adenosine. When guanosine-8-^{14}C was used for labeling, none of the pteridines isolated were labeled, an observation which indicated the loss of carbon 8 during conversion of the nucleoside to the pteridine.

More recent studies (Burg and Brown, 1966, 1968; Brown, 1970; Shiota et al, 1970; Fan and Brown, 1976) have led to the isolation of a single protein, or multiprotein aggregate, with a molecular weight of approximately 250,000. This enzyme unit, designated GTP cyclohydrolase (Burg and Brown, 1968), or dihydroneopterin triphosphate synthetase (Shiota et al, 1970), catalyzes the removal of carbon 8 from GTP by what appears to be a hydrolytic reaction. This carbon atom is recovered as formic acid. The other product of the reaction is dihydroneopterin triphosphate. Although theoretical considerations suggest that the formation of the initial pteridine intermediates would involve two hydrolytic steps, an Amadori rearrangement, and a final ring closure, all evidence indicates that the enzyme GTP cyclohydrolase catalyzes all these reactions (Burg and Brown, 1968; Wolf and Brown, 1969; Brown, 1970; Shiota et al, 1970). Recent in vivo and in vitro evidence for the presence of GTP cyclohydrolase and its enzymatic actions has been reported in several eye color mutants of D. melanogaster (Rasmusson et al, 1973; Fan et al, 1976).

The reaction scheme involved in the initial steps in biosynthesis of the pteridine ring in bacteria and insects has been elucidated as follows: (1) The starting material is GTP. (2) Carbon 8 is eliminated as formic acid and can be eliminated as CO_2 or can reenter the pathway of purine synthesis. (3) The intermediates are triphosphates. The initial intermediate is a hydrogenated and phosphorylated pyrimidine. (4) An Amadori rearrangement takes place. (5) On ring closure, the compound is converted to a non-fluorescent "initial pteridine" similar to tetrahydrobiopterin. (6) The first pteridine product appears to be 7,8-dihydroneopterin-3'-triphosphate, or a tetrahydrogenated carbon 6-substituted pterine (neopterin). (7) The formation of the side chain from ribose would be expected to be of the D-erythro configuration. Substances such as those described above have been isolated from insects as well as from bacterial systems (Rembold and Hanser, 1960; Goto and Forrest, 1961; Rembold and Buschmann, 1963, 1964; Watt, 1967; Shiota et al, 1970; Cone and Guroff, 1971). Unpublished results of Ziegler (Ziegler and Harmsen, 1969) indicate the presence of a number of such phosphorylated non-fluorescent compounds in the larvae and newly emerged adults of D. melanogaster.

Biosynthesis of the carbon 6-substituted pteridines is easily visualized since simple modification of the side chain of neopterin could readily give rise to biopterin, sepiapterin, isosepiapterin, and related pterines. Unfortunately, it is the carbon 6-substituted pteridines which have presented the greatest problems in interpretation. An objection to the hypothesis that carbon 6-substituted pteridines are derived from

neopterin is that the first stable intermediate should have a side chain with the D-erythro configuration, whereas biopterin has the L-erythro configuration (McLean et al, 1965). Results of feeding experiments and in vitro studies have indicated that an as yet unisolated epimerase is most likely responsible for inversions at carbon 1' and carbon 2' on the side chain (Jones and Brown, 1967; Brown, 1970). Feeding experiments in Drosophila (Brenner-Holzach and Leuthardt, 1959, 1961, 1967) showed that the carbon 6 side chain originated from glucose. Watt (1967), studying guanosine incorporation in the butterfly Colias eurytheme demonstrated that the sepiapterin side chain originated from the ribose moiety of guanosine. More recent evidence (Cone and Guroff, 1971; Dayman and Guroff, 1971) supports the hypothesis that the naturally occurring pterines are derived by a simple rearrangement of the neopterin side chain.

An alternative biosynthetic pathway for the naturally occurring carbon 6-substituted pteridines has been suggested in which carbon units are attached directly to pterine and/or 6-methylpterine. Forrest and Nawa (1962) have shown that it is possible to synthesize 6-alkylpterines such as biopterin, sepiapterin, isosepiapterin, and the drosopterins by reacting a non-alkylated pterine with a reactive propionaldehyde derived from threonine via α-ketobutyric acid. When a number of ^{14}C-labeled pteridines were fed to larvae of D. melanogaster, it was determined from labeling patterns in recovered pteridines that the original carbon 6 side chain had become detached and a new side chain attached in its place (Goto et al, 1964; Okada and Goto, 1965; Goto et al, 1965; Sugiura and Goto, 1967). Experiments examining incorporation of ^{14}C-labeled guanosine-5'-

phosphate in D. melanogaster (Sugiura and Goto, 1967) yielded patterns of incorporation of label into the pteridines similar to those reported for the butterfly Colias eurytheme (Watt, 1967). Goto et al (1964) also demonstrated in vivo incorporation of reduced pterine into the drosopterins, supporting the in vitro demonstration by Viscontini and Weilenman (1959) that red condensation products are produced upon oxidation of tetrahydrobiopterin.

Hadorn and Mitchell (1951) postulated that a genetic and biochemical relationship existed between a number of the fluorescent compounds found in D. melanogaster eyes and the red eye pigments. Ziegler (1960), on reinvestigating the sepia mutant of D. melanogaster, found large amounts of tetrahydrobiopterin (a tetrahydrogenated, non-fluorescing, labile pterine), which, when exposed to light, quickly was dehydrogenated to form pterine-6-carboxylic acid. Ziegler (1961), reexamining the results of an analysis of paper chromatograms of eye extracts prepared during development in various D. melanogaster wild type and mutant strains (Hadorn and Ziegler, 1958), found that only a limited number of pteridines were actually present, including tetrahydrobiopterin, sepiapterin, isosepiapterin, and isoxanthopterin. Other pteridines which had previously been isolated (pterine, pterine-6-carboxylic acid, and xanthopterin) were postulated to be degradation products formed during extraction and chromatography. Forrest and Nawa (1962) determined the structures of two additional dihydrogenated pteridines, sepiapterin and isosepiapterin, which have been shown to undergo slow oxidation to degradation products. Throckmorton (1962) reviewed briefly three previously proposed biosynthetic pathways

for the pteridines (simple pterines, carbon 6-substituted alkylpterines, and drosopterins) and proposed a fourth pathway based on the results of his comparative study of species of the genus Drosophila. Ziegler and Harmsen (1969) hypothesized that drosopterin synthesis probably involves dehydration of tetrahydrobiopterin via sepiapterin, but they did not rule out the possibility of parallel rather than sequential synthesis. The current conflicting alternatives to proposed biosynthetic pathways of the carbon 6-substituted polyhydroxyalkylpterines and drosopterins are each based on extensive experimental evidence, both in vivo and in vitro, and may simply reflect the existence of several alternative pathways available for the synthesis of these pteridines.

Biosynthesis of the simple carbon 6- and carbon 7-pterines, as well as the carbon 7-substituted alkylpterines, has been investigated in a number of laboratories (Weygand and Waldschmidt, 1955; Forrest et al, 1956; Ursprung and Hadorn, 1961; Weygand et al, 1961; Simon et al, 1963; Harmsen, 1963, 1964, 1966a,b,c, 1969, 1970; Forrest et al, 1966; Watt, 1967; Rembold and Gutensohn, 1968; Rembold et al, 1969; Rembold, 1970). In general terms, the simple pterines appear to be secondary products of conversion of the carbon 6-substituted polyhydroxyalkylpterines, formed through irreversible oxidative elimination of the carbon 6 sidechain (Rembold, 1970; Harmsen, 1970). Biosynthesis of the simple pterines appears to be equivalent to catabolism of the carbon 6-substituted pterines (Harmsen, 1970). Biosynthesis of the carbon 7-substituted alkylpterines appears to follow an alternative pathway secondary to production of the carbon 6 or carbon 7 simple pterines (Harmsen, 1963, 1964, 1966a,b,c,

1969, 1970; Forrest et al, 1966; Forrest,1970).

Forrest et al (1956) demonstrated in vivo synthesis of isoxanthopterin from 2-amino-4-hydroxypteridine in D. melanogaster via the action of xanthine oxidase. Previous studies of the biotransformation of xanthopterin to leucopterin (Krebs and Norris, 1949) and of 2-amino-4-hydroxypteridine to isoxanthopterin (Wieland and Liebig, 1944) had indicated the importance of xanthine oxidase in the biosynthesis of the simple carbon 6 and carbon 7 pterines.

Weygand and coworkers (Weygand and Waldschmidt, 1955; Weygand et al, 1961; Simon et al, 1963) studied the biosynthesis of leucopterin in butterflies and demonstrated its synthesis from purine precursors, but failed to demonstrate a specific mechanism of synthesis. Okada and Goto (1965) demonstrated in vivo conversion of ^{14}C-pterine to labeled isoxanthopterin in feeding experiments with larvae of D. melanogaster. Watt (1967) conducted the most extensive study to date on biosynthesis of simple pterines and the carbon 7-substituted alkylpterine erythropterin. He made use of the butterfly Colias eurytheme and a number of labeled compounds. Results of the study provided evidence for synthesis of leucopterin and erythropterin as end products of xanthopterin metabolism. The carbon 7 side chain has been shown to be secondarily added to xanthopterin in the synthesis of erythropterin (Forrest et al, 1966; Watt, 1967; Descimon, 1967). Watt (1967) demonstrated that the side chain of erythropterin can originate from lactate, malate, pyruvate, or serine. All available evidence indicates that synthesis of the carbon 7-substituted alkylpterines follows a pathway quite different from that of carbon

6-substituted alkylpterines.

Rembold and Gutensohn (1968) demonstrated that isoxanthopterin, xanthopterin, and leucopterin can be formed through oxidation at the carbon 6 and/or carbon 7 positions of 2-amino-4-hydroxypteridine under the influence of xanthine oxidase. More recent evidence indicates that oxidation of the carbon 6 position requires that the substrate exist as 7,8-dihydropteridines (Rembold and Gutensohn, 1968; Rembold et al, 1969; Rembold, 1970). The simple pterines appear to be synthesized in the fat body and then transported to their site of elimination or storage (Ursprung and Hadorn, 1961; Harmsen, 1966c). Harmsen (1969, 1970) found that the biosynthetic outcome of simple pterine synthesis in _in vivo_ systems can be affected by the partial pressure of oxygen to which the system is exposed. Descimon (1967), using pupae of _Colias croceus_, found that the state of reduction of pterine affects the product outcome for this compound. Thus, the same enzyme systems contained in different tissues under different oxygen tensions could be influenced either directly or indirectly in such a way that the metabolic expression of the final products is altered.

Since the biosynthetic pathway of GTP to neopterin or tetrahydrobiopterin has become generally accepted, the recent isolation of GTP cyclohydrolase (Brown, 1970; Shiota et al, 1970) and the postulated existence of an epimerase (Jones and Brown, 1967; Brown, 1970) have elucidated the metabolism of the initial pteridines. Mutant and wild type _D. melanogaster_ have proven invaluable models for studying the enzymatic reactions in anabolism and catabolism of the pteridines (Forrest et al, 1956; Hadorn and Schwinck, 1956; Glassman and Mitchell, 1959; Ursprung and Hadorn, 1961;

Munz, 1964). The xanthine oxidase system has been studied primarily in the rosy, maroon-like, and bronzy mutants of D. melanogaster (Forrest et al, 1956; Hadorn and Schwinck, 1956; Hadorn and Graf, 1958; Schwinck, 1965; Glassman, 1962, 1965, 1966). The groups of enzymes variously known as xanthine oxidases or xanthine dehydrogenases have been identified as responsible for oxidation of carbons 2, 4, and 7 of the pteridine nucleus (Wieland and Liebig, 1944; Krebs and Norris, 1949; Forrest et al, 1956; Bergman and Kwietny, 1959; Rembold and Gutensohn, 1968; Harmsen, 1970). The xanthine oxidase preparations appear to be non-selectively active for all three sites (Bergman and Kwietny, 1959). Oxidations at carbons 6 and 7 are of major importance in Drosophila. The reaction was first described for biotransformation of 2-amino-4-hydroxypteridine to isoxanthopterin (Wieland and Liebig, 1944; Lowry et al, 1949; Forrest et al, 1956) and of xanthopterin to leucopterin (Krebs and Norris, 1949). It appears that oxidation of carbon 7 is the final and nonreversible reaction in pteridine catabolism (Watt, 1967; Harmsen, 1970; Rembold, 1970). In Drosophila, xanthine oxidase activity is high in the larval fat body and in adult hemolymph (Ursprung and Hadorn, 1961; Munz, 1964). The peak period of activity of the enzyme in D. melanogaster occurs at 3 days after eclosion (Munz, 1964), with minimum activity in early pupae. Recent work (Harmsen, 1969, 1970; Rembold, 1970) indicates that xanthine oxidase also catalyzes oxidation of carbon 6, but only in the 7,8-dihydropterines. The experimental evidence suggests that carbon 6 oxidation versus carbon 7 oxidation by xanthine oxidase depends on the state of reduction of the substrate rather than on properties of the enzyme.

The mutants rosy, maroon-like, and bronzy in D. melanogaster have been shown to lack xanthine oxidase activity (Hadorn and Schwinck, 1956; Forrest et al, 1956), but when extracts of the mutants were exposed to antibodies to wild type xanthine oxidase, cross-reacting material was formed (Glassman and Mitchell, 1959). Thus, mutants which lack xanthine oxidase activity appear to lack some cofactor essential for enzymatic activity, as opposed to lack of the enzyme itself. Transplantation of xanthine oxidase-containing tissues or organs, such as fat body or Malpighian tubules, from wild type D. melanogaster to suitable mutants (e.g., brown) stimulates production of carbon 7-oxidized pteridines, as well as increasing drosopterin synthesis (Hadorn and Schwinck, 1956; Hadorn and Graf, 1958; Glassman and Mitchell, 1959; Glassman, 1966). The stimulation of drosopterin formation in mutants by tissue implants has been postulated to indicate a role of xanthine oxidase in drosopterin synthesis (Hadorn and Schwinck, 1956; Hadorn and Graf, 1958; Ursprung, 1961; Keller and Glassman, 1965; Boni et al, 1967).

Several workers have suggested that sepiapterin and a reduced biopterin derivative might be metabolically interconverted (Nathan and Ziegler, 1961; Taira, 1961a; Matsubara and Akino, 1964). This reaction was clearly demonstrated with the isolation of a specific sepiapterin reductase (Matsubara et al, 1966). Recognition of the biological importance of reactions involving the hydrogenation and dehydrogenation of carbon 6-substituted pterines is critical to an understanding of the biosynthetic pathways of the pteridines and the physiological roles of certain carbon 6-substituted pterines. Sepiapterin isolated from

Drosophila eyes is reduced both chemically and enzymatically with $NADPH_2$ serving as electron donor (Ziegler and Jaenicke, 1959; Taira, 1961a,b; Matsubara et al, 1963). A sepiapterin reductase has been isolated from Drosophila pupae which reduces the yellow sepiapterin to colorless dihydrobiopterin via reduction of the keto group on the side chain (Taira, 1961b). Two additional enzymes which have been studied in silkworms and are of metabolic significance throughout the Insecta are dihydrofolate reductase (Matsubara et al, 1963), which converts sepiapterin to a tetrahydro compound, and sepiapterin deaminase, which converts sepiapterin to a lumazine designated xanthopterin B2 (Aruga et al, 1954; Tsusue, 1967).

Forrest et al (1961) isolated an enzyme system from D. melanogaster which appears similar to xanthine oxidase except that it also catalyzes the conversion of 2-amino-4-hydroxypteridine to 2,4-dihydropteridine (lumazine). The conversion of pterines to lumazines is a clearly enzymatic reaction which cannot be interpreted as a simple chemical hydrolysis of the 2-amino group (Rembold, 1970).

In summary, two major stages in formation of the simple pterines from the carbon 6-substituted polyhydroxyalkylpterines are recognized: (1) cleavage or loss of the carbon 6 side chain and (2) partial or complete oxidation and dehydrogenation of 7,8-dihydropterine. An additional step may be inserted between (1) and (2), in which deamination of the pteridine ring occurs (Rembold, 1970). It can be concluded that the balance of products formed during pteridine catabolism depends upon the activity of pterine deaminases and xanthine oxidases and on the extent of reduction of the specific pterine substrate of each enzyme system.

CHAPTER II

MATERIALS AND METHODS

Experimental Organisms

Origin and Description

Organisms used in this study were five wild type stocks of Drosophila repleta collected originally in different geographical locations. One additional wild type stock was collected by the author and its relative success in pair matings and mass matings with the five stocks which had been in laboratory culture for extended periods of time was briefly studied.

The stocks of D. repleta studied included wild type stocks from Atlanta, Georgia (ar 4), Oahu, Hawaii (ar 5), Yucatan Peninsula, Mexico (ar 6), Palmar de Norte, Costa Rica (ar 11), and Sydney, Australia (ar 12). Dr. Ann M. Colley, Georgia Institute of Technology, Atlanta, Georgia, has maintained these stocks in laboratory culture since they were established in 1968 and 1970 from the collection of Dr. Anita I. Bolinger, Georgia State University, Atlanta, Georgia. The author has maintained separate cultures of these stocks since the origin of this project. The stock designated ar 1000 was collected by the author in 1974 at the Laboratory for Ophthalmic Research, Emory University, Atlanta, Georgia. Symbolic stock designations, except for ar 1000, are those of Dr. Bolinger. Origins and stock designations of these stocks are listed in Table 1.

Table 1. Symbolic Designations of Stocks of *D. repleta*

Symbolic Stock Designation	Collection Site	Source and Date Originally Obtained
ar 4	Atlanta, Georgia	Derived from a stock collected by A. M. Colley at Georgia State University in 1965 and 1966
ar 5	Oahu, Hawaii	Collected in Oahu, Hawaii in 1969; obtained from H. L. Carson, Washington University, Saint Louis, Missouri in 1970
ar 6	Yucatan Peninsula, Mexico	Obtained from L. H. Throckmorton, University of Chicago in 1970
ar 11	Palmar, Costa Rica	Obtained from Genetics Foundation, University of Texas, Austin, Texas in 1970
ar 12	Sydney, Australia	Obtained from Genetics Foundation, University of Texas, Austin, Texas in 1970
ar 1000	Atlanta, Georgia	Collected by the author at the Laboratory for Ophthalmic Research, Emory University, Atlanta, Georgia in 1974

Culture and Maintenance

Instant Drosophila Medium 67-5002 (Carolina Biological Supply Company), supplemented with Fleischmann's Active Dry Yeast, was used as the culture medium. Clean 8 oz Boston Round bottles with polypropylene foam plugs were sterilized by autoclaving at 15 lb pressure for 30 min and were allowed to cool before addition of medium and yeast. The medium was rehydrated with an equal volume of distilled water. Stock cultures were maintained by transfer at least monthly to fresh food bottles. Cultures were maintained at 23 $\pm$ 1 ^{o}C in a controlled temperature room. Shelves in this room were lined with "No Bugs M'Lady" Shelf and Drawer Paper to reduce the problem of mite infestation.

Developmental Studies

The first phase of the study was initiated by collecting and chromatographing eggs of each stock during successive 24 hr periods and following development of the organisms through the three larval instars and pupation to eclosion. Two to four sets of duplicate samples were prepared and analyzed for each day of development in each stock.

Oviposition Units

In the course of daily biochemical studies it became necessary to design and test several types of oviposition chambers in attempts to minimize the time of handling of parental flies and to obtain samples derived from eggs not older than 24 hr. Oviposition units were designed to provide the following conditions and information for each of the five stocks studied:

(1) Collection of a sufficient number of eggs of each stock to be utilized sequentially during each day of development in preparation of samples for qualitative determination of pteridine patterns and UV-absorbing compounds.

(2) Observation of each stock to estimate timing of daily developmental progress through the life cycle (i.e., timing of larval instar transformations, pupation, onset of eye and body coloration in pupae, and eclosion).

(3) Observation of morphological events in each stock which might be useful as markers to establish the approximate age of individual larvae and pupae and to determine any obvious phenotypic differences among stocks. The design of these units, designated Oviposition Units Type I, Type II, and Type III, is described in detail in Appendix A.

All three types of oviposition units were populated with adults collected from stock cultures. Adults were pooled, anesthetized with ethyl ether, and sexed and were added to oviposition chambers at an approximate ratio of one male to two females. Cultures used for collection of parents were aged at least five days from eclosion of the first adult in order to insure sexual maturity of parental adults (Ward and Stone, 1952; Colley, 1967). Adult males were allowed to remain in oviposition chambers to permit continued insemination of females and insemination of any virgin females which may have been collected.

Subculturing

Since total numbers of developing larvae in relation to amount of available food were observed to exert a distinct influence on the

duration of larval instars, petri dishes crowded with eggs or developing larvae were subcultured to fresh food dishes. Attempts were made to keep the total number of developing larvae to fewer than 200 organisms per 100 x 15 mm dish.

Timing of Stages of the Life Cycle and Morphological Characteristics

During collection and preparation of daily sequential samples of the five stocks of D. repleta for chromatographic studies, daily observations of developmental advances and morphological changes within and differences between stocks were routine. Eggs on oviposition surfaces and larvae developing from subcultured eggs could be observed readily throughout development with a dissecting microscope. Observations were recorded for each plate culture on successive days to determine times of initial appearance of each instar, pupation, eye coloration and adult body coloration within the pupal case, and eclosion. The dates recorded for larval molts for each instar were based on observed differences in mouth parts of each successive molt, coloration of posterior spiracles, presence of multiple mouth parts, and presence of molted mouth parts and exoskeleton of the previous instars in the medium. Third instar larvae were examined daily for changes in morphology and coloration of the anterior spiracles.

Adult Materials and Aging

In the second phase of the study, analyzing pteridine patterns of imagoes of selected ages dated from eclosion, a minimum of two sets of studies per stock using mass matings to obtain adults of specified age

were designed to provide the following conditions and information:

(1) Collection of sufficient numbers of adults of each sex per stock during 24 hr eclosion intervals to be used sequentially during aging for preparation of chromatograms of heads and bodies in determination of qualitative pteridine patterns of each stock and the extent of sexual dimorphism in pteridines and UV-absorbing compounds within each stock.

(2) Collection of enough adults of each sex to determine, by visual estimate and fluorimetric measurement, differences in isoxanthopterin concentration in bodies between stocks and changes within each stock during aging.

For each stock, 10-20 mass matings were set in 8 oz Boston Round bottles. Culture bottles were prepared in the same manner as for stock cultures. Approximately 40 pairs of adults (anesthetized, sexed, and randomly selected from stock cultures) were placed in each mass mating bottle. Flies were not precisely aged prior to use but were taken from bottles which had contained F_1 adults for at least 10 days. Mating and oviposition were allowed to continue for approximately seven days, after which the adults were transferred to fresh mass mating bottles for a second seven-day period. The seven-day mass mating interval was used to limit the total number of larvae competing for food sources, resulting in more uniform body weight among freshly eclosed flies.

Adults of each stock emerging within sequential 24 hr intervals were collected and classified as Eclosion Day 1 (hereafter designated E1, with each successive day of aging indicated by an "E" and the day

number). E1 adults were collected daily from all mass mating bottles set for a given stock and were pooled for aging. The E1 adults collected were anesthetized and sexes were separated. Male and female flies were aged separately in freshly prepared culture bottles with a maximum of 100 flies per bottle. Since differences were observed in patterns of pteridines and UV-absorbing compounds of virgin and inseminated females, it was decided to use virgin females for routine chromatographic analysis. Bottles used for aging of females were retained a minimum of 21 days (Humphrey, 1974) after use in chromatographic studies and were examined for evidence of developing larvae to confirm the virginity of females used for chromatography. Aging bottles were checked at minimum intervals of five days to insure that no culture conditions were developing which might adversely affect the aging flies (e.g., presence of developing larvae, excessive growth of yeast, mite or mold contamination, and excessive moisture). When any such detrimental condition arose in aging bottles, flies were discarded.

Interstock Crosses between ar 1000 and Other Stocks

During collection of adults for aging studies, it was convenient to perform a limited number of pair matings and mass matings between ar 1000 and the other stocks (ar 4, ar 5, ar 6, ar 11, and ar 12), among which extent of reproductive isolation has been analyzed (Humphrey, 1974). The purpose of this section of the study was to determine in a cursory manner the degree of success in interstock crosses between a freshly isolated wild type stock of D. repleta and stocks which had been in

laboratory culture for extended periods of time and to determine whether crosses with a low frequency of success in the previous study of these stocks (Humphrey, 1974) would be more or less successful if the Atlanta parental stock had been in laboratory culture for only a short time. Experiments included a maximum of four pair matings and two mass matings per cross, using five males and five females per cross.

Chromatographic Standards

Identification of fluorescent and UV-absorbing compounds isolated by chromatography was accomplished, where possible, by comparison with commercially available standards of pteridines, pteridine derivatives, kynurenine, kynurenine-related compounds, and a number of purine-based UV-absorbing compounds. Standards were used to determine ratio-to-front (Rf) values, characteristic colors of fluorescence, absorbance of short-wave ultraviolet light, and changes in absorbance or fluorescent color of compounds in basic or acidic environments (determined by exposure of dried chromatograms to ammonia or acetic acid vapors).

Pteridines and Pteridine Derivatives

Standards of known pteridines and pteridine derivatives were purchased from Sigma Chemical Company. Stock solutions of isoxanthopterin (0.002 g/ml), riboflavin (saturated solution), and other standards (0.004 g/ml) were prepared with 1.0 N NH_4OH as a solvent, except that leucopterin was dissolved in 0.1 N NaOH at a concentration of 0.004 g/ml. Abbreviations of standards, Rf values after development

in propanol and butanol solvents, color of fluorescence after completion of two-dimensional chromatography, and changes in fluorescence after exposure of dried chromatograms to ammonium hydroxide or acetic acid vapors are listed in Table 2. Identification of sepiapterin and isosepiapterin, which were not commercially available, was based on comparison of Rf values and other chromatographic properties with those of compounds isolated from the sepia mutant of D. melanogaster and comparison with reported Rf values and other characteristics of these compounds reported in the literature (Hadorn and Mitchell, 1951; Buzzati-Traverso, 1953; Hubby and Throckmorton, 1960; Throckmorton, 1962; Harmsen, 1966b; Colley, 1967; Howell, 1969).

For chromatography, standards were diluted to a concentration of 0.2 µg/µl in 1.0 N NH_4OH. A total volume equivalent to 1 µg of each standard, as measured with an Eppendorf pipette, was chromatographed.

Kynurenine and Kynurenine-Related Compounds

DL-kynurenine, kynurenic acid, xanthurenic acid, and anthranilic acid were purchased from Sigma Chemical Company. DL-kynurenine was prepared at a stock concentration of 0.004 g/ml in 0.1 N HCl. Kynurenic acid, xanthurenic acid, and anthranilic acid were prepared in 1.0 N NH_4OH at a stock concentration of 0.004 g/ml. Table 3 lists abbreviations, fluorescent colors, and Rf values of these compounds. Standards were diluted to a concentration of 0.2 µg/µl in 1.0 N NH_4OH for chromatography. As for pteridine standards, 1 µg of each standard was chromatographed.

Table 2. Chromatographic Characteristics of Standards: Pteridines and Pteridine Derivatives

Standard	Color of Fluorescence after Chromatography	Rf ± S.E. Propanol	Rf ± S.E. Butanol	N[1]
2-Amino-4-Hydroxypteridine	Blue	0.529 ± 0.0075	0.438 ± 0.0064	7
Biopterin	Blue	0.543 ± 0.0222	0.423 ± 0.0150	4
Folic Acid[2]	UV-Absorbing at 265 nm	0.202 ± 0.0150	0.398 ± 0.0059	4
Isosepiapterin	Dull Yellow	0.580 ± 0.0041	0.527 ± 0.0037	6
Isoxanthopterin	Violet	0.272 ± 0.0047	0.340 ± 0.0035	9
Leucopterin[3]	White to Pale Blue	0.097 ± 0.0053	0.149 ± 0.0041	7
Pterine-6-Carboxylic Acid	Sky Blue	0.295 ± 0.0098	0.266 ± 0.0048	7
Riboflavin	Dull Yellow	0.492 ± 0.0096	0.390 ± 0.0075	7
Sepiapterin	Yellow	0.446 ± 0.0059	0.427 ± 0.0052	6
Xanthopterin[4]	Green	0.255 ± 0.0056	0.410 ± 0.0032	9

[1]Number of Determinations.

[2]Folic acid takes on a sky-blue fluorescence after sitting, possibly representing pterine-6-CH_3, pterine-6-COOH, or another product of folic acid degradation.

[3]Leucopterin turns sky-blue when exposed to NH_3 vapors, then slowly reverts to original color.

[4]Xanthopterin turns yellow when exposed to acetic acid vapors.

Table 3. Chromatographic Characteristics of Standards: Kynurenine and Kynurenine-Related Compounds

Standard	Color of Fluorescence after Chromatography	Rf ± S.E. Propanol	Rf ± S.E. Butanol	N[1]
Anthranilic Acid	Violet	0.685 ± 0.0197	0.902 ± 0.0350	4
Kynurenic Acid	Green	0.681 ± 0.0097	0.536 ± 0.0278	4
DL-Kynurenine	Light Blue-Green	0.651 ± 0.0099	0.459 ± 0.0374	4
Xanthurenic Acid[2]	Blue	0.461 ± 0.0234	0.487 ± 0.0140	4

[1]Number of Determinations.

[2]Xanthurenic acid upon sitting after two-dimensional chromatography becomes colorless under UV light. When exposed to NH_3 vapors and UV light, the compound takes on a characteristic yellow fluorescence which quickly disappears. Exposure of freshly isolated xanthurenic acid to NH_3 vapors also results in a change in fluorescent color from blue to yellow, followed by fading back to the original blue fluorescence.

Purine-Based UV-Absorbing Compounds

In view of the limited data on UV-absorbing compounds available from previous chromatographic studies of Drosophila (see references in section on pteridine standards), the postulated importance of certain purine compounds in the proposed biosynthetic pathways of pteridines (Weygand and Waldschmidt, 1955; Brenner-Holzach and Leuthardt, 1959, 1961, 1967; Watt, 1967), the pathways of nitrogenous waste products in the genus Drosophila, and the surprisingly large number of previously unrecorded UV-absorbing compounds observed in this study, a number of purine-based standards were studied chromatographically in an attempt to identify as many of the unknowns as possible. Purine-based UV-absorbing compounds used as standards are listed in Table 4.

Chromatographic Methods

Two-dimensional ascending paper chromatography was used as the method of separation for qualitative analysis of pteridines and UV-absorbing compounds. One-dimensional chromatograms developed in the propanol solvent were eluted for quantitative measurement of isoxanthopterin in adults. Chromatographic separations were carried out in a standard sandwich chamber (Brinckman Instruments, Inc.) using Whatman No. 1 Chromatography Paper (W. R. Balsten, Ltd.) as the chromatographic medium. Reported methods for preparation of chromatograms for the analysis of pteridines and UV-absorbing compounds in Drosophila and methods for ascending chromatography (Hadorn and Mitchell, 1951; Buzzati-Traverso, 1953; Throckmorton, 1962; Strickberger, 1964; Harmsen, 1966b; Woods and Waitzman, 1970) were modified for the sandwich chamber.

Table 4. Chromatographic Characteristics of Standards: Purine-Based UV-Absorbing Compounds

Standards[1,2]	Rf ± S.E. Propanol	Butanol	N[3]
Adenine	0.608 ± 0.0132	0.543 ± 0.0151	4
Adenosine	0.652 ± 0.0123	0.436 ± 0.0084	4
5'-Adenosine Monophosphate	0.298 ± 0.0063	0.122 ± 0.0035	6
Adenosine Triphosphate	0.171 ± 0.0173	0.027 ± 0.0035	4
Guanine	0.413 ± 0.0183	0.388 ± 0.0083	3
Guanosine Triphosphate	0.124 ± 0.0093	0.020 ± 0.0008	4
Hypoxanthine	0.580 ± 0.0080	0.461 ± 0.0087	8
Inosine	0.574 ± 0.0079	0.356 ± 0.0094	2
Inosine Monophosphate	0.230 ± 0.0081	0.071 ± 0.0019	6
Uric Acid	0.356 ± 0.0108	0.320 ± 0.0063	8
Xanthine	0.497 ± 0.0072	0.429 ± 0.0083	3
Xanthosine	0.462 ± 0.0126	0.320 ± 0.0047	2

[1]For purposes of chromatography, standards were diluted to 0.2 µg/µl in 1N NH_4OH.
[2]All compounds are UV-absorbing at 265 nm.
[3]Number of Determinations.

Chromatographic Medium

Whatman No. 1 Chromatography Paper was used throughout the study as the chromatographic medium, without pretreatment, standardization to a constant relative humidity, or solvent equilibration. All chromatographic studies were done using sheets cut to measure 18 x 18 cm, with two exceptions: (1) The first analysis of daily biochemical changes in fluorescent and UV-absorbing compounds employed a wick method, described below; and (2) several preliminary chromatographic series comparing times of solvent runs, resolution, and reproducibility of ascending and descending chromatography used sheets measuring 18 x 30 cm.

Standard 46 x 57 cm sheets provided by the manufacturer were cut to the desired dimensions, note being taken of the machine direction of the original sheet. Sheets of chromatography paper were always handled with steel forceps or protective paper to reduce the possibility of contamination during preparation and were stored between layers of Saran Wrap or Glad Wrap until used.

Types of Chromatograms

(a) Standard Long Sheets: Standard long sheets were cut to measure 18 x 30 cm with the machine direction running the length of the paper. Ten pencil marks were placed 3 cm from the lower edge of the sheet and spaced 1.5 cm apart with a side margin of 2.0 cm. These sheets were used to obtain preliminary comparative data for the five stocks of D. repleta and to compare time of solvent run, resolution of compounds, and reproducibility in ascending and descending chromatographic developments. From results of this preliminary study, it was

determined that resolution by ascending chromatography was as good as that obtained by descending chromatography with comparable time of solvent run for sheets of this size. It was also determined that reproducibility of Rf values was considerably more consistent with ascending development in sandwich chambers than with descending chromatography.

(b) Wicked Sheets: Wicked sheets were used only in the initial series of daily developmental analyses. Wicked sheets were cut to the standard sheet size of 18 x 18 cm, with note being taken of the machine direction of the paper, so that the first chromatographic development was parallel to the machine direction. Pencil marks were placed 2 cm from the lower edge of the sheet and spaced along the sheet at 3 cm intervals starting from the left hand side. Samples to be chromatographed were prepared by methods to be discussed later. The sheets were placed in sandwich chambers and developed in the machine direction for 3 1/2 hr at 37 ^{o}C in the dark with the propanol solvent. Sheets were then air-dried a minimum of 24 hr and viewed with a hand-held ultraviolet lamp. The widths of the tracks of the five samples were measured and were cut to provide wicks measuring either 2.5 x 18 cm or 3.0 x 18 cm (depending on the spread of the sample during its development). From a standard sheet of chromatography paper, sheets were cut measuring 15.8 x 18 cm or 15.3 x 18 cm, with the machine direction running the width of the sheets. The wick was placed on the undeveloped sheet with 3 mm overlap to provide a composite sheet measuring 18 x 18 cm. The composite sheets were then developed in the standard manner using the

butanol solvent in the second dimension. After development, the sheets were air-dried a minimum of 24 hr in the dark and were examined under ultraviolet light sources for viewing of isolated fluorescent and UV-absorbing compounds.

(c) Standard Sheets: Standard 18 x 18 cm sheets were used for all studies except those previously described. Sheets of Whatman No. 1 paper were prepared, making note of the machine direction. With the machine direction having been indicated in the top right-hand corner of the sheet, a pencil mark was made 2 cm from the lower edge and 2 cm from the left margin. The sample was applied to the sheet on the pencil mark. The first chromatographic development was always run in the propanol solvent, in the machine direction, under standard conditions and was dried as described above. After drying, the sheet was rotated 90 degrees, so that compounds separated by the first chromatographic development were perpendicular to the direction of the second development, which was run against the machine direction of the paper using the butanol solvent. Chromatograms were air-dried in the dark until viewed with a multi-band ultraviolet light source.

Preparation and Standard Conditions for Chromatography

Daily sequential samples of developmental stages were prepared according to a modification of procedures of Hadorn and Mitchell (1951) and Throckmorton (1962). In the initial series samples were not weighed. Instead, a constant number of larvae and pupae were used for each stock per sample per day (eggs = 500; D1-2 = 400; D3 = 200; D4 = 50; D5 = 25; D6 = 10; D7-15 = 5).

Developmental stages (eggs, larvae, or pupae) were collected at sequential 24 hr intervals from food-containing petri dishes on which females had been allowed to oviposit or to which larvae had been subcultured. Samples were transferred to Drosophila Ringer solution (Ephrussi and Beadle, 1936), washed free of adherent Drosophila medium, and drained on wetted filter paper. They were then washed twice in deionized water and drained. At this point, the lighting in the room was reduced to that provided by a shielded incandescent lamp.

In the initial developmental series using wicked sheets, samples were placed in a boiling water bath for 3 min to coagulate proteins and were removed and placed on dry filter paper. After removal of adhering water, samples were transferred to the corresponding origin of the chromatogram and crushed with the clean, smooth end of a glass rod. Due to the large volume of the boiling water bath (25 ml) relative to volume occupied by samples, retrieval of samples was quite time-consuming. It was necessary to continue to boil samples since unboiled samples of developmental stages showed extensive trailing of pteridines in the first dimension, as reported by Hadorn and Mitchell (1951). In the case of adult heads, the small amount of protein relative to pteridines did not interfere with chromatographic development. Hadorn and Mitchell (1951) reported only a small loss of material from whole D. melanogaster during boiling. However, in the present study, when larval samples of D. repleta were placed in a glass scintillation vial containing approximately 0.5 ml deionized water and boiled for 3 min, a visible yellow color was imparted to the water. Hadorn and Mitchell did not specify

the exact conditions of the experiments which they conducted to determine loss of material or the type of samples used. If their determinations were based entirely on studies of adult flies, the discrepancy is explainable since this author found negligible differences in quantities of pteridines when adult samples were boiled in water and when they were dry-boiled.

Dry-boiling is defined by this author as the use of a dry 1.5 ml Eppendorf reaction tube (polypropylene with attached cap) to hold the samples during immersion for 5 min in a boiling water bath. Except for the initial developmental series on wicked sheets described above and one series of samples consisting of adult heads of each sex in each stock which were crushed directly on the chromatography sheets after decapitation (5 heads per sample), all chromatograms were prepared using the dry-boiling technique.

After eggs, larvae, and pupae were washed and drained as described above, the samples were weighed on a Mettler balance (Model H64) and transferred to 1.5 ml Eppendorf reaction tubes which were sealed and placed in a boiling water bath for 5 min. The samples were then removed and chromatograms were prepared as described above. Weighed samples represented numbers of eggs, larvae, or pupae chromatographed in the initial daily developmental series.

Adults were sampled at days E1, E5, E10, and E15 and were anesthetized, weighed, and dry-boiled for 5 min. In each series of two-dimensional analyses for qualitative studies, 5 flies per sample per sex per stock were used. In quantitative studies, 10 replications of one

fly per sample per sex per stock were used. The flies were removed from the Eppendorf reaction tube and decapitated with a clean razor blade. Heads and bodies were then crushed separately on chromatography paper as described above.

To protect chromatograms from contamination and to reduce loss of material during crushing of samples, a piece of clean waxed paper was placed under each chromatogram prior to sample application. All chromatograms were stored between sheets of waxed paper in a light-excluding container and allowed to dry for at least 24 hr prior to chromatography.

Solvents

Two chromatographic solvents were used in this study, one of basic pH and the other of acidic pH. All components of solvents were Fisher reagents meeting or exceeding A. C. S. standards for purity. The basic solvent (propanol solvent) was n-propanol:ammonium hydroxide:deionized water, 8:1:3, v/v/v (Throckmorton, 1962). The propanol solvent was prepared immediately prior to use, was the first solvent in all two-dimensional runs, and was used as the only solvent in the isolation of isoxanthopterin from samples of adult bodies for quantitative studies. The acidic solvent (butanol solvent), which consisted of n-butanol:glacial acetic acid:deionized water, 20:3:7, v/v/v (Strickberger, 1964), was used for all developments in the second dimension. The butanol solvent was prepared 24-48 hr before use.

Preparation of Sandwich Chambers

The ascending chromatography sandwich chamber (Brinckman Instruments, Inc.) consisted of two main parts: (1) a stainless steel trough-

support unit and (2) a sandwich chamber constructed of glass and teflon. Two glass plates were separated by 7 mm side and top teflon spacers (kindly provided by the Laboratory for Ophthalmic Research, Emory University, Atlanta, Georgia) to replace the 4 mm spacers supplied with the commercial unit. The sandwich chambers were prepared for chromatographic developments according to the standard method described below. The chamber was assembled with the back glass plate separated from the front glass cover by the spacers. On each side of the chamber two pieces of tape (Time Tape Company) were placed to run from the back plate across the side spacer to the front cover. An additional piece of tape was similarly placed at the top of the unit. The back of the chamber was sealed with tape overlapping the length of contact between glass and teflon.

At this time, all lights were turned off except for a shielded incandescent lamp adjusted to provide indirect lighting of low intensity. The front cover plate of the chamber was removed and a 16 x 18 cm piece of teflon matting (VWR Scientific) was placed on the inner surface of the back plate. Chromatograms to be developed were then brought from darkened storage areas and protective covers were removed. Using steel forceps, chromatograms were placed on the teflon matting in the desired orientation to the direction of solvent development to follow. Next, a second piece of teflon matting was placed over the first chromatogram, followed by a second chromatogram, with the chromatograms aligned appropriately with respect to each other and to the lower edge of the back glass plate. Finally, another piece of teflon matting was placed over

the second chromatogram and the cover plate was replaced. The sides were retaped and sealed along areas of contact. The sealed sandwich chamber was then placed in the trough of the trough-support unit and covered with aluminum foil to protect the chromatograms from additional light exposure during handling of the chamber and during solvent runs.

Development of Chromatograms

Initially, chromatograms were developed in a 37 °C controlled temperature room, until this room became unavailable for further use. All further studies were conducted in an unlighted controlled temperature incubator (Model 300, Fisher Scientific Company) set at 37 °C.

Under greatly reduced lighting conditions, the sandwich chamber-trough-support unit was placed in the 37 °C incubation chamber. The aluminum foil cover was removed and the sandwich chamber was set aside. Fifty ml of solvent were added to the trough of the trough-support unit. The sandwich chamber was then immediately replaced in the trough and was sealed with tape at all metal-to-glass contact points. The aluminum foil was replaced, and the chromatograms were allowed to develop. Development times for each solvent were standardized to 3 1/2 hr per solvent system.

Removal, Drying, Viewing, and Storage of Chromatograms

At the completion of each chromatographic development, the sandwich chambers were removed from the incubator and disassembled in a completely darkened room. The chromatograms were removed with steel forceps and were placed to dry in a polyethylene tray which had its bottom surface lined with teflon matting. The chromatograms were

air-dried for 1-7 days prior to preparation for second dimensional chromatography or viewing of isolated compounds with one of two types of multi-band ultraviolet light source, either a ChromatoVue Cabinet (Fisher Scientific Company) or a hand-held ultraviolet lamp MineraLight UVSL 25, Ultra Violet Products). The two principal emissions for both sources were 2540 and 3660 μm. After drying, chromatograms were sealed between two sheets of Glad Wrap and stored in the dark for subsequent viewing or elution for quantitative measurement.

Fluorimetric Quantitation of Isoxanthopterin

Quantitation of isoxanthopterin in male and female bodies of the five stocks of D. repleta at four selected imaginal ages (E1, E5, E10, and E15) was accomplished by use of a Model 111 Fluorometer (F. K. Turner). The primary excitation filter was a Kodak 18A glass filter (transmission from 300-400 nm, with peak percent transmission at 366 nm). The secondary filter was a Turner No. 405 (peak percent transmission at 405 nm). In those cases in which attenuation of the fluorescence of a sample was required, use was made of the window settings of the fluorometer (1X, 3X, 10X, and 30X) and/or a Kodak 10% neutral density filter was employed.

Quantitative determinations of unknowns were begun by cutting the isoxanthopterin spot from the chromatogram of each sample in a dark room using a short-wave ultraviolet lamp as the source of illumination. Each spot was cut into small pieces and then was placed in a 13 x 100 mm screw-cap Kimax tube. After addition of 5 ml 1.0 N NH_4OH to each

sample tube, tubes were sealed and mixed for 30 sec on a Vortex hand mixer. After mixing, samples were centrifuged using an International EXD centrifuge at a dial setting of 50 (approximately 1600 rpm). The sample tubes were removed from the centrifuge, wiped clean with a moistened Kimwipe tissue, and then wiped dry. Tubes were then transferred to the fluorometer fitted with the standard door attachment and were read. Tubes were calibrated by matching against a standard tube arbitrarily selected as a zero fluorescence blank, which allowed for correction of variations in fluorimetric readings of samples due to variability in transmission properties and background fluorescence of different tubes.

Extraction and quantification of isoxanthopterin using the above procedure was verified by extraction of isoxanthopterin standard (Sigma Chemical Company) at known concentrations (0.001, 0.05, 0.1, 0.5, and 1.0 μg/ml in 1.0 N NH_4OH). Results indicated a minimum of 99% recovery of eluted standard when compared to the same concentration of standard added directly to the solvent; therefore, no correction for loss of material during extraction or continued adherence to the chromatography paper after elution was employed.

Standard curves for each window setting and filter arrangement were determined using concentrations of isoxanthopterin standard ranging from 1.0×10^{-4} to 1.0 μg/ml. The isoxanthopterin concentration of each unknown sample was determined by comparison of fluorimetric readings with corresponding readings on the standard curves.

The fluorometer was zeroed at each setting using a 1N NH_4OH blank and was checked against tubes containing 1, 2, 3, and 4 cm^2 pieces of chromatography paper cut from a blank sheet which had been developed in the basic solvent. The maximum difference in fluorescence between the NH_4OH blank and any of the chromatographic blanks was never greater than 0.5 divisions, which is approximately equivalent to the normal oscillation of the dial (0.25-0.33 units) observed when taking readings. Thus, correction for differences in surface area of paper for different unknown samples was considered unnecessary.

Ten samples of each sex were used for each stock to determine isoxanthopterin concentration in bodies on days E1, E5, E10, and E15, with a total of 400 fluorimetric determinations of isoxanthopterin concentration for all stocks collectively. Fluorimetric readings for the ten individual samples of each sex per stock per day were recorded and converted to µg/ml NH_4OH using the standard curves. Isoxanthopterin concentration was expressed as µg/mg wet weight of the individual sample. The mean concentration of isoxanthopterin ($\pm$ standard error), expressed as µg/mg wet weight, was calculated for males and females of each stock on days E1, E5, E10, and E15. Interstock differences and intrastock sexual differences in mean isoxanthopterin concentration and relationships between isoxanthopterin concentration and imaginal age were analyzed by the unpaired small-sample Student t test, confidence limit analysis, and least square linear regression analysis, according to methods outlined by Snedecor and Cochran (1967) and Goldstein (1967).

CHAPTER III

RESULTS

Cross-Fertility of ar 1000Q with ar 4, ar 5, ar 6, ar 11, and ar 12

Cross-fertility of ar 1000 with ar 4, ar 5, ar 6, ar 11, and ar 12 was examined by setting a maximum of four pair matings and two mass matings (five males and five females) per cross. A successful pair mating or mass mating was defined as a mating producing adult offspring. Table 5 summarizes the results of pair and mass matings.

In crosses between the recently isolated Atlanta wild type stock ar 1000 and the established laboratory cultures of ar 4 (Atlanta), ar 5 (Hawaii), and ar 6 (Yucatan), successful pair and mass matings were achieved in each case. $F_1 \times F_1$ crosses were set using the pooled offspring of the pair matings and all crosses were observed to be fertile.

All crosses between ar 1000 and either ar 11 (Costa Rica) or ar 12 (Australia) were unsuccessful whether in pair matings or mass matings. In both pair and mass matings for these crosses, eggs were observed in fairly large numbers. At no time were first instar larvae or any later developmental stages observed.

Morphological Events during the Life Cycle

A brief summary of observations of timing of developmental stages and associated morphological events in each of the five stocks is presented in Table 6. During collection of eggs for chromatographic

Table 5. Cross-Fertility of ar 1000 (Recently Isolated Atlanta Stock) with ar 4 (Atlanta), ar 5 (Hawaii), ar 6 (Yucatan), ar 11 (Costa Rica), and ar 12 (Australia)

Interstock Cross	ar 1000 Males x Females of				
	ar 4	ar 5	ar 6	ar 11	ar 12
Success in Pair Matings	+	+	+	-	-
Success in Mass Matings	+	+	+	-	-

Interstock Cross	ar 1000 Females x Males of				
	ar 4	ar 5	ar 6	ar 11	ar 12
Success in Pair Matings	+	+	+	-	-
Success in Mass Matings	+	+	+	-	-

+ = Successful

- = Not Successful

Table 6. Timing of Stages of the Life Cycle in Five Stocks of *Drosophila repleta*

Stage or Event	Day of Development (from Oviposition)				
	ar 4	ar 5	ar 6	ar 11	ar 12
Egg/Embryo	0-2	0-1	0-1	0	0
First Instar Larva	2-3	2-3	2-3	1-3	1-3
Second Instar Larva	4-5	4-5	4-5	3-4	3-4
Third Instar Larva	5-9	5-9	5-9	4-8	4-8
Pupation	9-10	9-10	9-10	8-9	8-9
Onset of Reddish-Brown Eye Coloration (Ommochrome Deposition)	13	13	13	12	12
Onset of Adult Body Coloration	14	14	14	13	13
Eclosion	15-16	15-16	15-16	14-15	14-15
Total No. Days from Oviposition to Eclosion	15-16	15-16	15-16	14-15	14-15

studies, it was observed that eggs of all stocks except ar 12 (Australia) have a distinctive yellow color. The eggs of all stocks are elongated and have four long filaments, as is characteristic of the species. The distinctive off-white coloration of ar 12 eggs, as opposed to the yellowish coloration in other stocks, is also characteristic of larval instars.

Differences in timing of embryogenesis from oviposition to hatching of the first larval instar were distinctive and provided a basis for groupings of the stocks. Ar 11 and ar 12 first instar larvae were consistently found on egg-laying plates after a 24-hour oviposition period (day 0), while ar 5 and ar 6 first instar larvae consistently appeared between day 0 and day 1. Cultures of these stocks checked at 36 hours consistently had first instar larvae present, whereas plates checked at 30 hours had few to none present. Ar 4 first instar larvae consistently did not appear until the closing hours of day 2.

While ar 4 appears to have a longer period of embryogenesis than ar 5 or ar 6, the interval of development to the second instar molt was consistently the same for these three stocks, as was timing of all other developmental stages of the life cycle (Table 6). The ar 11 and ar 12 stocks were observed to spend a greater period of time in the first instar larval stage than did the other stocks, and a shorter period of time in the second instar. They remained 24-48 hours ahead of other stocks in the timing of subsequent developmental stages, as well as in total time from oviposition to eclosion.

In all stocks prior to molting of each instar, new mouth parts (i.e., mouth hooks) were visible prior to loss of the previous set. Molting of the exoskeleton did not appear to be necessary for loss of the previous instar mouth parts since ejection or loss of the previous set prior to molting of the exoskeleton was observed in a number of larval studies. Molting of the exoskeleton of the previous instar involves emergence of the next instar through an opening in the posterior section of the exoskeleton, during which the old exoskeleton appears to be turned inside out.

Morphological Markers of Developmental Events

First instar larvae of all stocks were found to have a distinctive black band encircling each of the two posterior spiracles just anterior to its openings. Second instar larvae developed a brownish-red band around the posterior spiracles in the same location in which the black band had previously appeared. Thus, the distinctive differences in mouth parts of each instar, relative size of the instars, and coloration of the posterior spiracles are morphological markers which make it possible to distinguish first and second instar larvae. Transition from first to second instar, as well as from second to third instar, can be determined by the presence of duplicate sets of mouth hooks.

Soon after the molt leading to the third instar, all stocks begin to develop a black band at the base of the anterior spiracles, development of this band being completed within 24 hours. During the last day in the second instar, the anterior spiracles appear to take on the shape

of a spadelike "fin." Prior to this time the anterior spiracles are simple paired tubes ending in a pair of narrow single openings, with little if any morphological distinction.

No later than the day following entry into the third instar, the anterior spiracles of all stocks have a number of finger-like extensions, each terminating in a black tip. During the next 24-hour period of development, the area of the anterior spiracle between the tip of each extension and the band at the common base of the anterior spiracle takes on a charcoal gray color.

Approximately 24 hours prior to pupation, the anterior spiracles of ar 4, ar 5, ar 6, and ar 11 take on a brownish-red color. Anterior spiracles of ar 12, by contrast, never develop a brownish-red coloration. In all other stocks, the onset of brownish-red coloration of the anterior spiracles is an excellent marker heralding the onset of pupation since within 24-36 hours of its appearance the third instar larvae undergo pupation. Since ar 12 does not develop the reddish-brown coloration, it is not possible to use this characteristic as a marker for expected time of pupation.

All stocks showed a preference for pupation on the sides of the bottles or on dry surfaces of the petri dishes, but in all stocks a number of third instar larvae were found to pupate in or on the surface of the food. In all stocks, as pupation began, the anterior spiracles were fully extended and the finger-like extensions were spread. In all stocks the exoskeleton of third instar larvae became the outer covering of the pupa, whose color ranged from amber to dark brown.

Approximately three days after pupation in all stocks, differentiation of imaginal eyes is distinctly visible within the puparium. At this time, the eyes are reddish-brown. The reddish-brown color is attributed to the presence of ommochromes since chromatographic studies did not reveal the presence of drosopterins at this time. Approximately 24 hours after the onset of reddish-brown coloration of the eyes, all stocks begin to take on adult body coloration. Deposition of melanin and other pigments responsible for adult body color appears to start at the head and to proceed posteriorly through the thorax to the abdomen.

The body color of all stocks at the time of eclosion is pale gray which progressively darkens to the deep grayish-brown adult coloration characteristic of the species during a period of 24-48 hours in all stocks except Costa Rica. The body color of the Costa Rican stock (ar 11) remains distinctly lighter than in other stocks. The eyes are scarlet in all stocks at eclosion. By the fifth day after eclosion, eyes of ar 4, ar 5, and ar 6 have developed the sepia color characteristic of the species, while ar 12 eyes develop a brick-red coloration. The eye color of ar 11 does not darken detectably with age, but remains bright-red, or scarlet, throughout adult life.

Chromatographic Characteristics of Fluorescent and UV-Absorbing Compounds Isolated during Development and Aging

Abbreviations used to identify each of the fluorescent and UV-absorbing compounds isolated during two-dimensional chromatographic analysis of the five stocks of *D. repleta* during development and aging are listed in Tables 7-9. The abbreviations are presented to simplify

Table 7. Abbreviations of Identifiable Fluorescent Compounds Isolated from Adult Heads and Bodies and Developmental Stages

Abbreviation	Fluorescent Color	Compound
AHP-BIO	Blue	2-Amino-4-Hydroxypteridine and/or Biopterin
DRO I	Yellow-Orange	Drosopterin I
DRO II	Orange	Drosopterin II
DRO III	Orange	Drosopterin III
ISOSP	Dull Yellow	Isosepiapterin
ISOX	Blue-Violet	Isoxanthopterin
KA	Green	Kynurenic Acid
KYN	Blue	Kynurenine
P-6-CH_3	Blue	6-Methylpterine
P-6-COOH	Blue	Pterine-6-Carboxylic Acid
RIB	Yellow	Riboflavin
SP	Yellow	Sepiapterin
XAN	Green	Xanthopterin
XIC	Blue	Xanthurenic Acid

Table 8. Abbreviations of Unidentified Fluorescent Compounds Isolated from Adult Heads and Bodies and Developmental Stages

Abbreviation	Fluorescent Color	Isolation Source
BB	Blue	Adult Bodies
BH	Blue	Adult Heads
BY I	Burnt Yellow	1st Instar Larvae-Eclosion
BY II	Burnt Yellow	1st-Early 3rd Instar Larvae
G I	Green	Adult Bodies of Stocks ar 4, ar 5, and ar 6
G II	Green	1st-Early 3rd Instar Larvae
P I	Purple	All Developmental Stages, Adult Heads and Bodies
P II	Purple	1st-3rd Instar Larvae
P III	Purple	All Developmental Stages, Adult Heads and Bodies
P IV	Purple	Adult Bodies on Day E1
WB	White-Blue	1st Instar Larvae-Eclosion, Adult Bodies
W I	White	1st-Early 3rd Instar Larvae
W II	White	1st-Early 3rd Instar Larvae
W III	White	1st-Early 3rd Instar Larvae

Table 8. Abbreviations of Unidentified Fluorescent Compounds Isolated from Adult Heads and Bodies and Developmental Stages, Continued

Abbreviation	Fluorescent Color	Isolation Source
Y I	Yellow	Eggs, 3rd Instar Larvae, Pupae within 24 hr of Eclosion, Adult Heads
Y II	Yellow	Pupae within 24 hr of Eclosion, Adult Bodies
Y III	Yellow	Pupae within 24 hr of Eclosion, Adult Bodies
Y IV	Yellow	1st-3rd Instar Larvae
Y V	Yellow	Adult Heads

Table 9. Abbreviations of UV-Absorbing Compounds Isolated from Adult Heads and Bodies and Developmental Stages

Abbreviation	Compound
AD-A	Adenine and/or Adenosine
5'-AMP	5'-Adenosine Monophosphate
ATP-GTP	Adenosine Triphosphate and/or Guanosine Triphosphate
GUAN-XA	Guanine and/or Xanthine
HXA	Hypoxanthine
IMP	Inosine Monophosphate
UA	Uric Acid
UV I	Unidentified UV-Absorbing Compound Isolated from First, Second, and Early Third Instar Larvae
UV II	Unidentified UV-Absorbing Compound Isolated from First and Second Instar Larvae

the identification of the isolated compounds as represented in all following tables and figures.

The mean ratio-to-front (Rf) values (± standard error of the mean) for compounds isolated during this study are listed in Tables 10-12. The Rf values were determined from examination of 30 chromatograms which had been developed by the standard two-dimensional chromatographic procedure (development with propanol solvent, air-drying, development with butanol solvent).

A generally greater solute mobility in the propanol solvent was observed for commercial standards of pteridines and pteridine-related compounds (Table 2) than for compounds isolated during developmental and aging studies of the five stocks of D. repleta (Table 10). Greater mobility of commercial standards was not observed during the second dimensional development in the butanol solvent. Reduced mobility of the pteridines isolated from D. repleta during propanol solvent chromatography probably reflects the combined influence of binding phenomena and interference with migration by associated non-pteridine material.

Comparison of Rf values of the compounds chromatographically isolated from heads and from bodies and developmental stages (Tables 10-12) reveals a greater mobility of the solutes in chromatograms of heads than in chromatograms of bodies. The differences in Rf values for heads and bodies probably reflect the greater quantity of interfering non-pteridine material relative to pteridine content in bodies than in heads. While there are minor differences in mean Rf values for standards and for compounds isolated from heads, bodies, and developmental stages, the

Table 10. Rf Values of Identifiable Fluorescent Compounds Isolated During Development and Aging

Compound	Rf Propanol ± S.E. Bodies	Rf Propanol ± S.E. Heads	Rf Butanol ± S.E. Bodies	Rf Butanol ± S.E. Heads
AHP-BIO	0.454 ± 0.0059	0.476 ± 0.0054	0.454 ± 0.0041	0.455 ± 0.0035
DRO I		0.023 ± 0.0015		0.057 ± 0.0020
DRO II		0.034 ± 0.0018		0.103 ± 0.0023
DRO III		0.069 ± 0.0026		0.173 ± 0.0048
ISOSP	0.506 ± 0.0153	0.555 ± 0.0054	0.543 ± 0.0063	0.566 ± 0.0046
ISOX	0.177 ± 0.0041	0.209 ± 0.0067	0.344 ± 0.0066	0.358 ± 0.0078
KA	0.687 ± 0.0118	0.715 ± 0.0093	0.520 ± 0.0070	0.529 ± 0.0066
KYN	0.653 ± 0.0063		0.479 ± 0.0033	
P-6-CH_3	0.177 ± 0.0040		0.442 ± 0.0036	
P-6-COOH	0.204 ± 0.0082	0.214 ± 0.0043	0.260 ± 0.0030	0.272 ± 0.0049
RIB	0.394 ± 0.0056		0.422 ± 0.0036	
SP	0.371 ± 0.0063	0.385 ± 0.0051	0.456 ± 0.0036	0.463 ± 0.0040
XAN	0.236 ± 0.0064	0.266 ± 0.0065	0.345 ± 0.0042	0.362 ± 0.0042
XIC	0.313 ± 0.0079	0.406 ± 0.0051	0.516 ± 0.0048	0.532 ± 0.0036

Table 11. Rf Values of Unidentified Fluorescent Compounds Isolated during Development and Aging

Compound	Rf Propanol ± S.E. Bodies	Rf Propanol ± S.E. Heads	Rf Butanol ± S.E. Bodies	Rf Butanol ± S.E. Heads
BB	0.201 ± 0.0039		0.035 ± 0.0024	
BH		0.106 ± 0.0034		0.049 ± 0.0017
BY I	0.367 ± 0.0089		0.471 ± 0.0054	
BY II	0.499 ± 0.0074		0.559 ± 0.0070	
G I	0.362 ± 0.0089		0.484 ± 0.0046	
G II	Origin		Origin	
P I	0.727 ± 0.0038	0.737 ± 0.0034	0.428 ± 0.0040	0.444 ± 0.0038
P II	0.371 ± 0.0092		0.083 ± 0.0033	
P III	0.915 ± 0.0058		0.939 ± 0.0046	
P IV	0.214 ± 0.0092		0.242 ± 0.0062	
WB	0.437 ± 0.0048		0.138 ± 0.0041	
W I	0.131 ± 0.0032		0.090 ± 0.0043	
W II	0.420 ± 0.0074		0.197 ± 0.0076	
W III	0.249 ± 0.0076		0.246 ± 0.0082	

Table 11. Rf Values of Unidentified Fluorescent Compounds Isolated during Development and Aging, Continued

Compound	Rf Propanol ± S.E.		Rf Butanol ± S.E.	
	Bodies	Heads	Bodies	Heads
Y I	0.141 ± 0.0029		0.041 ± 0.0016	
Y II	0.126 ± 0.0048		0.160 ± 0.0035	
Y III	0.275 ± 0.0078		0.178 ± 0.0021	
Y IV	0.133 ± 0.0047		0.169 ± 0.0095	
Y V		0.275 ± 0.0046		0.400 ± 0.0046

Table 12. Rf Values of UV-Absorbing Compounds Isolated during Development and Aging

Compound	Rf Propanol ± S.E. Bodies	Heads	Rf Butanol ± S.E. Bodies	Heads
AD-A	0.593 ± 0.0091		0.485 ± 0.0044	
5'-AMP	0.189 ± 0.0036	0.220 ± 0.0050	0.118 ± 0.0027	0.123 ± 0.0028
ATP-GTP	Origin		Origin	
GUAN-XA	0.417 ± 0.0059		0.393 ± 0.0059	
HXA	0.520 ± 0.0133		0.493 ± 0.0083	
IMP	0.118 ± 0.0042	0.158 ± 0.0043	0.033 ± 0.0019	0.038 ± 0.0014
UA	0.272 ± 0.0045	0.323 ± 0.0099	0.325 ± 0.0032	0.342 ± 0.0086
UV I	0.011 ± 0.0016		0.196 ± 0.0073	
UV II	0.466 ± 0.0076		0.158 ± 0.0052	

relative positions of all identified compounds on each type of chromatogram are such that their identification is not in question. Variation in the location of each isolated compound is more evident after development in the propanol solvent and appears to be directly related to the amount of interfering material present.

Figure 2 is a composite chromatogram illustrating relative positions of the major fluorescent compounds isolated from the five stocks of D. repleta after two-dimensional development in the propanol and butanol solvents. Figure 3 is a composite chromatogram illustrating relative positions of UV-absorbing compounds isolated after two-dimensional development in the same solvents. Symbols used for the visually estimated quantities of fluorescent and UV-absorbing compounds isolated during development and aging are listed in Table 13.

Fluorescent and UV-Absorbing Compounds Isolated during Development

Abbreviations for the developmental stages of D. repleta used to summarize the results of the daily developmental chromatographic study are listed in Table 14. The visually estimated quantities of fluorescent and UV-absorbing compounds isolated from each stock at each stage of development are presented in Tables 15-20. Visually estimated quantities for each day of development in each stock appear in Appendix B, Tables 1-10.

The developmental timing of qualitative and quantitative fluctuations in fluorescent and UV-absorbing compounds in each stock appears to reflect the stock-specific timing of morphological events during

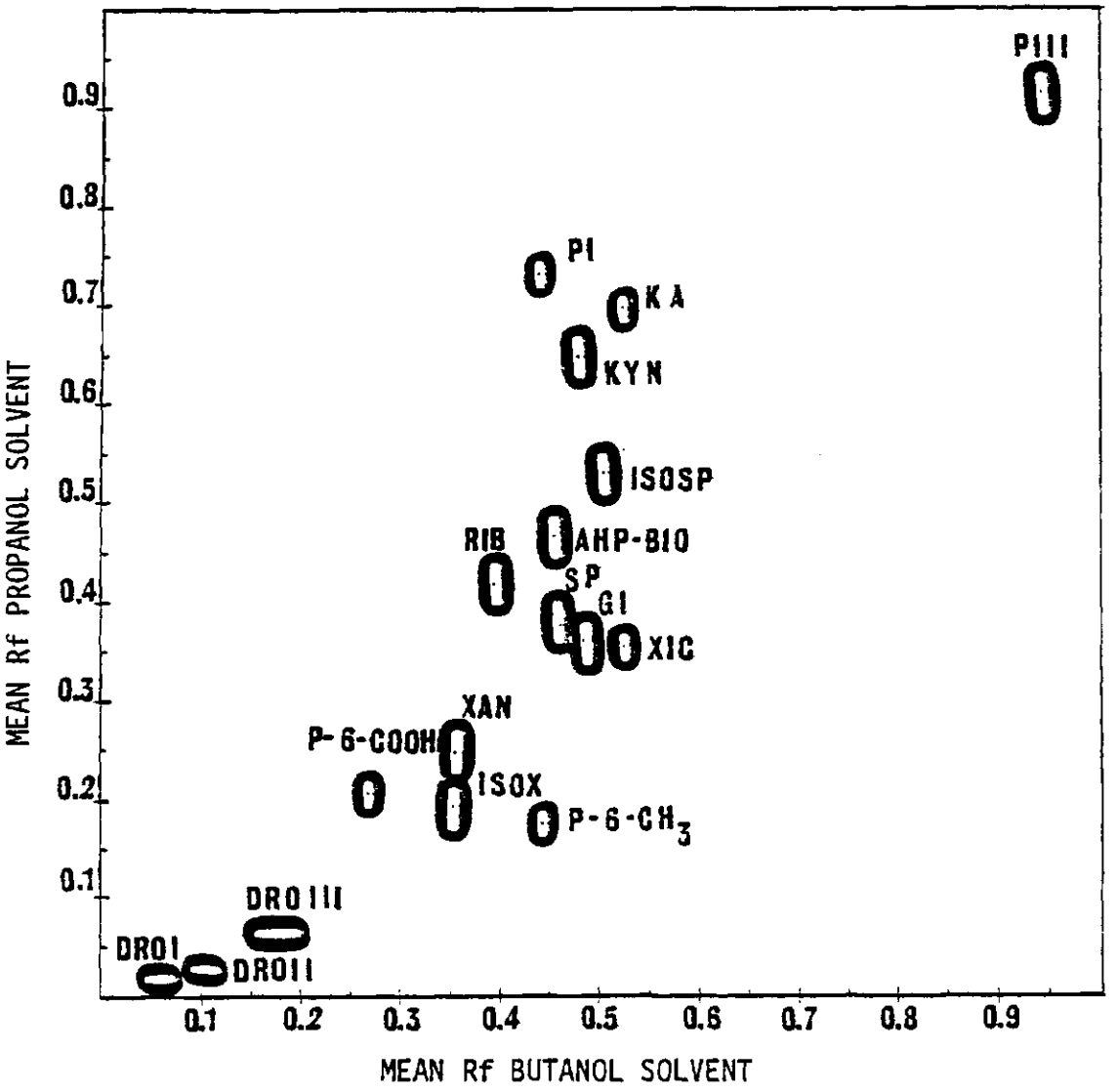

Figure 2. Composite Chromatogram Illustrating Relative Positions of Major Fluorescent Compounds After Two-Dimensional Development in Propanol and Butanol Solvent Systems

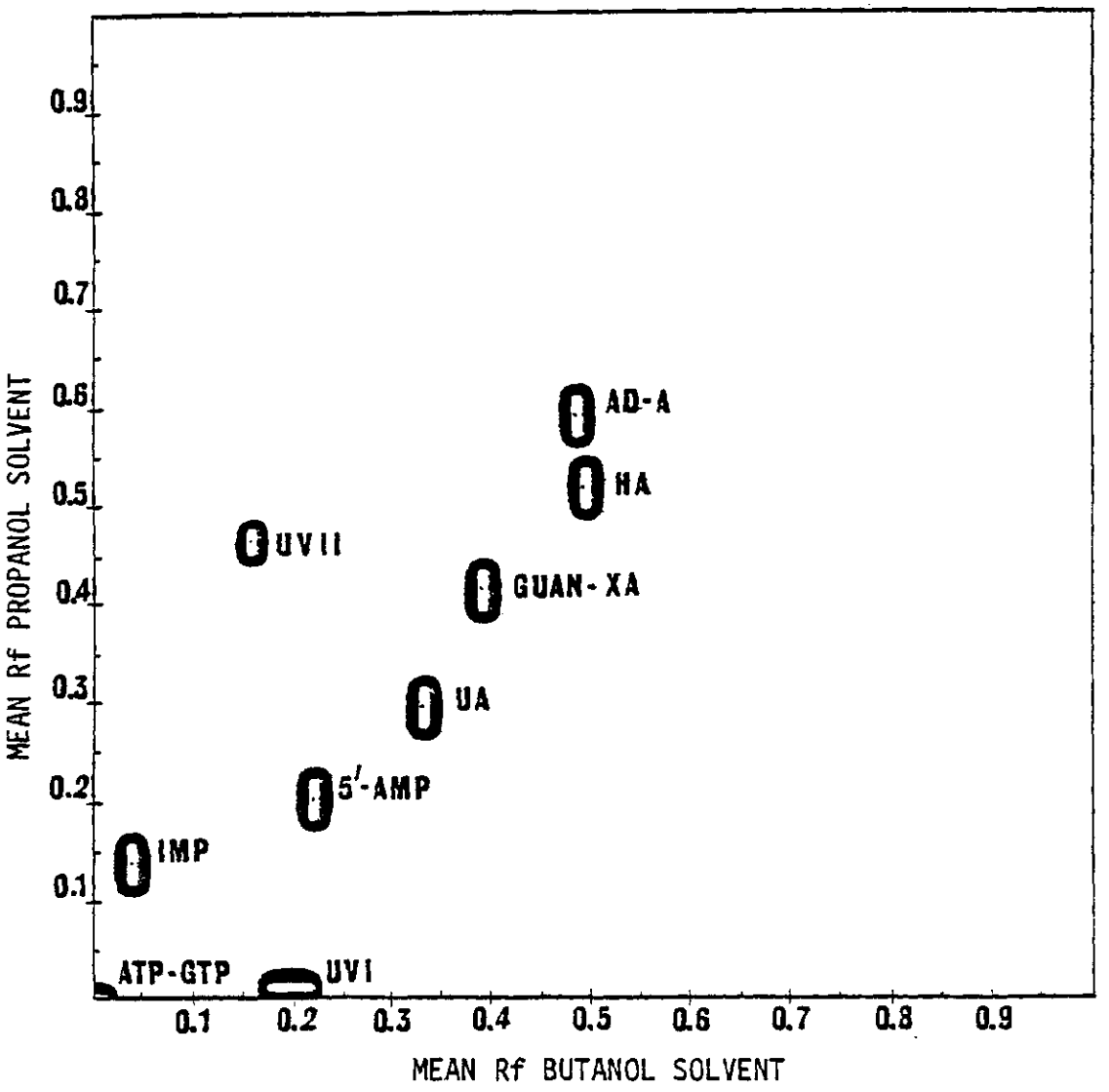

Figure 3. Composite Chromatogram Illustrating Relative Positions of UV-Absorbing Compounds After Two-Dimensional Development in Propanol and Butanol Solvent Systems

Table 13. Symbols for Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated during Development and Aging

Symbol	Quantity
0	None
±	Slight Trace
+	Trace
++	Trace-Small
+++	Small
++++	Small-Moderate
+++++	Moderate
++++++	Large
+++++++	Very Large

Table 14. Abbreviations of Developmental Stages in the Life Cycle of *Drosophila repleta*

Abbreviation	Developmental Stage or Event
Egg	Egg
L_1	1st Instar Larva
L_2	2nd Instar Larva
L_{3E}	Early 3rd Instar Larva
L_{3L}	Late 3rd Instar Larva
P_1	Onset of Pupation
P_2	Differentiation of Imaginal Eyes and Deposition of Ommochromes
P_3	Deposition of Pigments Responsible for Adult Body Coloration
P_4	Pupae within 24 Hours of Eclosion

Table 15. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 4, ar 5, and ar 6

Compound	Egg	L_1	L_2	L_{3E}	L_{3L}	P_1	P_2	P_3	P_4
AHP-BIO	0	+	±	+	+	±	++	++	+++
DRO I	0	0	0	0	0	0	0	+	+++
DRO II	0	0	0	0	0	0	0	0	+
DRO III	0	0	0	0	0	0	0	+	++++
ISOSP	0	0	0	0	0	0	0	0	±
ISOX	±	+	±	±	+	±	++	++	++
KA	±	+	+	±	±	+	+	+	+
KYN	±	++	+++	+++	+++++	+++	++++	++++	++++
P-6-CH_3	0	+	+++	+++	+	++	++++	++++	++++
P-6-COOH	0	0	0	0	0	0	0	0	+
RIB	±	++	++++	+++++	++++	+++	+++	++	+
SP	0	0	0	0	0	0	0	0	±
XAN	±	+	+	+	+	+	+	+	+
XIC	0	0	±	+	+	+	+	+	+

Table 15. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 4, ar 5, and ar 6, Continued

Compound	Egg	L_1	L_2	L_{3E}	L_{3L}	P_1	P_2	P_3	P_4
BY I	0	+++	+	+	±	+	+	+	±
BY II	0	++	±	±	0	0	0	0	0
G II	±	+++	+	±	0	0	0	0	0
P I	0	+	+	+	+	+	+	+	+
P II	0	±	++	+	±	0	0	0	0
P III	±	+	+++	++	+	++	+++	++	++
WB	0	++	++	++	±	±	+	+	+
W I	0	+	+	+	0	0	0	0	0
W II	0	+	±	±	0	0	0	0	0
W III	0	+	+	+	0	0	0	0	0
Y I	±	0	0	+	+	0	0	0	+
Y II	0	0	0	0	0	0	0	0	±
Y III	0	0	0	0	0	0	0	0	±
Y IV	0	+	+	+	0	0	0	0	0

Table 16. Visually Estimated Quantities of UV-Absorbing Compounds Isolated from Developmental Stages of ar 4, ar 5, and ar 6

Compound	Egg	L_1	L_2	L_{3E}	L_{3L}	P_1	P_2	P_3	P_4
AD-A	0	0	±	±	0	0	±	0	±
5'-AMP	+	+++	+++	++	±	±	+	+	+
ATP-GTP	+++	++++	++++	++++	+	+	+	±	±
GUAN-XA	0	++	+++	+++	±	±	++	++	+
HXA	0	0	+	+	0	+	0	±	±
IMP	+	+++	+++	++	±	0	0	0	+
UA	±	+++	++++	++++	+	+	++	++	+++
UV I	0	++++	+++	±	0	0	0	0	0
UV II	0	±	±	0	0	0	0	0	0

Table 17. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 11

Compound	Egg	L_1	L_2	L_{3E}	L_{3L}	P_1	P_2	P_3	P_4
AHP-BIO	0	+	±	+	+	±	++	+++	++++
DRO I	0	0	0	0	0	0	0	+	+++
DRO II	0	0	0	0	0	0	0	0	+
DRO III	0	0	0	0	0	0	0	+	++++
ISOSP	0	0	0	0	0	0	0	0	0
ISOX	±	++	+	+	++	+	+++	+++	+++
KA	±	+	+	+	+	+	+++	++	+
KYN	±	++	+++	+++	+++++	+++	+++	++	+
P-6-CH_3	0	+	+	+++	+	++	+++	+	+
P-6-COOH	0	0	0	0	0	0	0	0	0
RIB	±	++	+++	++++	+++	++	++	±	0
SP	0	0	0	0	0	0	0	0	0
XAN	±	+	+	+	+	+	+	+	+
XIC	0	0	±	±	+	+	+++	+	+

Table 17. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 11, Continued

Compound	Egg	L_1	L_2	L_{3E}	L_{3L}	P_1	P_2	P_3	P_4
BY I	0	+++	++	+	±	±	+	+	±
BY II	0	++	++	±	0	0	0	0	0
G II	±	+++	+	±	0	0	0	0	0
P I	0	+	+	+	+	+	+	+	+
P II	0	+	++	++	0	0	0	0	0
P III	±	+	+++	+++	+	++	+++	+++	++
WB	0	++	+++	++	±	±	±	±	+
W I	0	+	+	+	0	0	0	0	0
W II	0	+	+	+	0	0	0	0	0
W III	0	+	+	+	0	0	0	0	0
Y I	±	0	0	+	+	0	0	0	+
Y II	0	0	0	0	0	0	0	0	0
Y III	0	0	0	0	0	0	0	0	0
Y IV	0	+	+	+	0	0	0	0	0

Table 18. Visually Estimated Quantities of UV-Absorbing Compounds Isolated from Developmental Stages of ar 11

Compound	Egg	L_1	L_2	L_{3E}	L_{3L}	P_1	P_2	P_3	P_4
AD-A	0	0	±	±	0	0	±	0	±
5'-AMP	+	+++	++++	+++	±	+	+	+	+
ATP-GTP	+++	++++	++++	++++	+	+	+	±	±
GUAN-XA	0	++	+++	+++	±	++	+	+	±
HXA	0	0	+	+	0	+	0	±	±
IMP	+	+++	+++	++	±	0	0	0	+
UA	0	+++	++++	++++	++	++	+++	+++	+++
UV I	0	++++	+++	±	0	0	0	0	0
UV II	0	±	±	0	0	0	0	0	0

Table 19. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 12

Compound	Egg	L_1	L_2	L_{3E}	L_{3L}	P_1	P_2	P_3	P_4
AHP-BIO	0	+	+	+	+	±	++	+++	++++
DRO I	0	0	0	0	0	0	0	+	+++
DRO II	0	0	0	0	0	0	0	0	+
DRO III	0	0	0	0	0	0	0	+	++++
ISOSP	0	0	0	0	0	0	0	0	0
ISOX	±	++	+	+	++	++	++++	++++	++++
KA	±	+	±	±	±	+	++	++	+
KYN	±	++	+++	++	++	++	++++	++	+
P-6-CH_3	0	+	+	+++	+	++	+++	++	+
P-6-COOH	0	0	0	0	0	0	0	0	0
RIB	±	+	++	+++	++	++	++	+	0
SP	0	0	0	0	0	0	0	0	0
XAN	±	+	+	+	+	+	+	+	+
XIC	0	0	±	±	+	+	++	+	+

Table 19. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 12, Continued

Compound	Egg	L_1	L_2	L_{3E}	L_{3L}	P_1	P_2	P_3	P_4
BY I	0	+++	+	+	±	±	+	+	±
BY II	0	++	+	±	0	0	0	0	0
G II	±	+++	+	±	0	0	0	0	0
P I	0	+	+	+	+	+	+	+	+
P II	0	+	++	+	0	0	0	0	0
P III	±	+	+++	+++	+	+	+++	+++	++
WB	0	++	+++	++	±	±	±	±	+
W I	0	+	+	+	0	0	0	0	0
W II	0	+	+	+	0	0	0	0	0
W III	0	+	+	+	0	0	0	0	0
Y I	±	0	0	+	+	0	0	0	+
Y II	0	0	0	0	0	0	0	0	0
Y III	0	0	0	0	0	0	0	0	0
Y IV	0	+	+	+	0	0	0	0	0

Table 20. Visually Estimated Quantities of UV-Absorbing Compounds Isolated from Developmental Stages of ar 12

Compound	Egg	L_1	L_2	L_{3E}	L_{3L}	P_1	P_2	P_3	P_4
AD-A	0	0	±	±	0	0	±	0	±
5'-AMP	+	+++	++++	+++	±	+	+	+	+
ATP-GTP	+++	++++	++++	++++	+	+	+	±	±
GUAN-XA	0	++	+++	+++	±	++	+	+	±
HXA	0	0	+	+	0	+	0	±	±
IMP	+	+++	+++	++	±	0	0	0	+
UA	0	+++	++++	++++	++	++	+++	+++	+++
UV I	0	++++	+++	±	0	0	0	0	0
UV II	0	±	±	0	0	0	0	0	0

development. Thus, the patterns of timing of both morphological and biochemical events in the five stocks appear to be quite similar, although the specific day of appearance or disappearance of a given compound may vary among stocks due to differences in timing of developmental stages.

As indicated in Tables 15-20, the eggs of all five stocks contained slight trace amounts of isoxanthopterin, xanthopterin, riboflavin, kynurenine, kynurenic acid, and unknown yellow (Y I), purple (P III), and green (G II) fluorescent compounds. Eggs of all stocks also contained trace amounts of UV-absorbing compounds identified as inosine monophosphate and 5'-adenosine monophosphate and small amounts of ATP-GTP. One additional UV-absorbing compound identified as uric acid was found in eggs of ar 4, ar 5, and ar 6 at slight trace levels. Uric acid was not detected in either ar 11 or ar 12.

In first instar larvae all compounds present in eggs increased from slight trace to trace or from trace to small amounts, except for Y I, which disappeared. First instar larvae of all stocks also contained trace amounts of the folic acid breakdown product P-6-CH_3, AHP-Biopterin, P I (a purple fluorescent compound which may be an artifact), and seven unknown white and yellow fluorescent compounds (W I, W II, W III, WB, Y IV, BY I, and BY II). The only interstock differences in quantities of fluorescent compounds isolated during the first instar larval stage were a greater amount of isoxanthopterin and a smaller amount of riboflavin in ar 12 than in ar 4, ar 5, ar 6, and ar 11. All stocks showed a general increase in ATP-GTP from the small

quantities found in eggs to a small-moderate amount, as well as an increase from trace to small amounts of IMP and 5'-AMP. In addition to the UV-absorbing compounds found in eggs, first instar larvae of all stocks had small amounts of uric acid and a small-moderate amount of an unknown UV-absorbing compound (UV I) chromatographically unlike any of the purine-based standards which were studied. One additional UV-absorbing compound (UV II), which appears chromatographically similar to cyclic 3',5'-adenosine monophosphate (Rf values not reported), was observed at slight trace levels in all stocks.

In second instar larvae of all stocks the pattern of fluorescent compounds was similar to that in first instar larvae with only a few exceptions (Tables 15, 17, and 19). Second instar larvae of ar 11 and ar 12 continued to have a greater amount of isoxanthopterin (trace) than in stocks ar 4, ar 5, and ar 6 (slight trace). Riboflavin increased in all stocks, but ar 12 still had smaller amounts than in other stocks. Xanthurenic acid was present for the first time in all stocks at slight trace levels. The folic acid breakdown product P-6-CH_3 increased from trace to small amounts in ar 4, ar 5, and ar 6, while no increase in this compound occurred in ar 11 and ar 12. The unknown compounds W II, BY I, and BY II decreased in all stocks, while the purple-fluorescing compound P III increased in all stocks. The patterns and amounts of UV-absorbing compounds in the second instar were similar to those found in the first instar, with the exception of a reduction in the unknown UV I, which completely disappeared by early third instar (Tables 16, 18, and 20). Additionally, all stocks had trace amounts of hypoxanthine,

which had not appeared previously.

The patterns of fluorescent compounds in early third instar larvae were not qualitatively different from those of second instar larvae. Quantitatively, the folic acid breakdown product P-6-CH_3 increased in ar 11 and ar 12 to the same level as was found earlier in ar 4, ar 5, and ar 6. Kynurenic acid decreased to slight trace levels in all stocks except ar 11, as did the unknown G II. The UV-absorbing compounds present in early third instar larvae of all stocks were qualitatively and quantitatively similar to those of second instar larvae except that the cyclic AMP-like compound (UV II) completely disappeared. In late third instar larvae a number of the unknown fluorescent compounds (G II, W I, W II, W III, and Y IV) disappeared, and the quantities of all UV-absorbing compounds were reduced. The only fluorescent compound which increased was kynurenine. Riboflavin decreased in all stocks.

At the onset of pupation, quantities of isoxanthopterin, uric acid, 5'-AMP, and guanine-xanthine were higher in ar 11 and ar 12 than in ar 4, ar 5, and ar 6. All stocks showed a slight rise in P-6-CH_3 and a reduction in AHP-Biopterin, and riboflavin was reduced in all but ar 12.

At the time in pupation when the everted eyes have taken on a reddish-brown coloration, ar 11 and ar 12 showed distinct differences in quantity of fluorescent and UV-absorbing compounds when compared to ar 4, ar 5, and ar 6. The ar 11 and ar 12 stocks had higher levels of isoxanthopterin, xanthurenic acid, and kynurenic acid. The levels of P-6-CH_3 and guanine-xanthine were lower in ar 11 and ar 12 than in the other stocks. AHP-Biopterin increased slightly in all stocks.

In all stocks at the time during the pupal stage at which adult head coloration becomes detectable, trace levels of drosopterin I and drosopterin III were present. Quantities of riboflavin and P-6-CH_3 were smaller in ar 11 and ar 12 than in ar 4, ar 5, and ar 6, and amounts of AHP-Biopterin, isoxanthopterin, and kynurenic acid were greater in ar 11 and ar 12 than in the other stocks.

Pupae of all stocks just prior to eclosion had trace amounts of drosopterin II and the unknown Y I, small amounts of drosopterin I, and small-moderate amounts of drosopterin III. In ar 11 and ar 12, riboflavin disappeared and AHP-Biopterin increased to small-moderate amounts, while stocks ar 4, ar 5, and ar 6 maintained trace amounts of riboflavin and small amounts of AHP-Biopterin. In addition, ar 4, ar 5, and ar 6 had slight trace quantities of sepiapterin and isosepiapterin, trace amounts of P-6-COOH, and small-moderate amounts of P-6-CH_3 and kynurenine, while ar 11 and ar 12 had no P-6-COOH, sepiapterin, or isosepiapterin and trace amounts of P-6-CH_3 and kynurenine. UV-absorbing compounds were qualitatively and quantitatively similar in all stocks.

Fluorescent and UV-Absorbing Compounds Isolated from Adult Heads

Visually estimated quantities of fluorescent and UV-absorbing compounds isolated from heads of adult males and females of the five stocks of D. repleta at days E1, E5, E10, and E15 are presented in Tables 21-25. Qualitative and quantitative differences in fluorescent and UV-absorbing compounds in heads of males and females of each stock during aging were slight, as were differences among stocks.

Table 21. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 4 Adult Male and Female Heads at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	+++	++++	+++++	+++++	+++	++++	+++++	+++++
DRO I	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
DRO II	+	++	+++	+++	+	++	+++	+++
DRO III	+++++	++++++	+++++++	+++++++	+++++	++++++	+++++++	+++++++
ISOSP	+	++	++	++	+	++	++	++
ISOX	+	+	+	+	+	+	+	+
KA	+	0	0	0	+	0	0	0
KYN	+	0	0	0	+	0	0	0
P-6-COOH	+	++	+++	+++	+	++	+++	+++
SP	++	+++	+++	+++	++	+++	+++	+++
XAN	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
XIC	+	+++	+++	+++	+	+++	+++	+++
BH	+	+	+	+	+	+	+	+
P I	+	+	+	+	+	+	+	+
P III	+	0	0	0	+	+	+	+
Y V	+	+	+	+	+	+	+	+
5'-AMP	+	+	+	+	+	+	+	+
GUAN-XA	+	+	+	+	+	+	+	+
IMP	+	+	+	+	+	+	+	+
UA	+++	+++	++++	++++	+++	+++	++++	++++

Table 22. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 5 Adult Male and Female Heads at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	+++	++++	+++++	+++++	+++	++++	+++++	+++++
DRO I	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
DRO II	+	++	+++	+++	+	++	+++	+++
DRO III	+++++	++++++	+++++++	+++++++	+++++	++++++	+++++++	+++++++
ISOSP	+	++	++	++	+	++	++	++
ISOX	+	+	+	+	+	+	+	+
KA	+	0	0	0	+	0	0	0
KYN	+	0	0	0	+	0	0	0
P-6-COOH	+	++	+++	+++	+	++	+++	+++
SP	++	+++	+++	+++	++	+++	+++	+++
XAN	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
XIC	+	+	+++	++++	+	+	+++	++++
BH	+	+	+	+	+	+	+	+
P I	+	+	+	+	+	+	+	+
P III	+	0	0	0	+	+	+	+
Y V	+	+	+	+	+	+	+	+
5'-AMP	+	+	+	+	+	+	+	+
GUAN-XA	+	+	+	+	+	+	+	+
IMP	+	+	+	+	+	+	+	+
UA	+++	+++	+++	+++	+++	+++	+++	+++

Table 23. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 6 Adult Male and Female Heads at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	+++	++++	+++++	+++++	+++	++++	+++++	+++++
DRO I	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
DRO II	+	++	+++	+++	+	++	+++	+++
DRO III	+++++	++++++	+++++++	+++++++	+++++	++++++	+++++++	+++++++
ISOSP	+	++	++	++	+	++	++	++
ISOX	+	+	+	+	+	+	+	+
KA	+	0	0	0	+	0	0	0
KYN	+	0	0	0	+	0	0	0
P-6-COOH	+	++	+++	+++	+	++	+++	+++
SP	++	+++	+++	+++	++	+++	+++	+++
XAN	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
XIC	+	+++	+++	+++	+	+++	+++	+++
BH	+	+	+	+	+	+	+	+
P I	+	+	+	+	+	+	+	+
P III	+	0	0	0	+	+	+	+
Y V	+	+	+	+	+	+	+	+
5'-AMP	+	+	+	+	+	+	+	+
GUAN-XA	+	+	+	+	+	+	+	+
IMP	+	+	+	+	+	+	+	+
UA	+++	+++	++++	++++	+++	+++	++++	++++

Table 24. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 11 Adult Male and Female Heads at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	+++	++++	+++++	+++++	+++	++++	+++++	+++++
DRO I	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
DRO II	+	++	+++	+++	+	++	+++	+++
DRO III	+++++	++++++	+++++++	+++++++	+++++	++++++	+++++++	+++++++
ISOSP	+	++	++	++	+	++	++	++
ISOX	++	++	++	++	++	++	++	++
KA	+	+	+	+	+	+	+	+
KYN	+	+	+	+	+	+	+	+
P-6-COOH	+	+	+	+	+	+	+	+
SP	++	+++	+++	+++	++	+++	+++	+++
XAN	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
XIC	+	+	+++	++++	+	+	+++	++++
BH	+	+	+	+	+	+	+	+
P I	+	+	+	+	+	+	+	+
P III	+	+	+	+	+	+	+	+
Y V	+	+	+	+	+	+	+	+
5'-AMP	+	+	+	+	+	+	+	+
GUAN-XA	+	+	+	+	+	+	+	+
IMP	+	+	+	+	+	+	+	+
UA	+++	+++	+++	+++	+++	+++	+++	+++

Table 25. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 12 Adult Male and Female Heads at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	+++	++++	+++++	+++++	+++	++++	+++++	+++++
DRO I	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
DRO II	+	++	+++	+++	+	++	+++	+++
DRO III	+++++	++++++	+++++++	+++++++	+++++	++++++	+++++++	+++++++
ISOSP	+	++	++	++	+	++	++	++
ISOX	+++	+++	+++	+++	+++	+++	+++	+++
KA	+	0	0	0	+	0	0	0
KYN	+	0	0	0	+	0	0	0
P-6-COOH	+	+	+	+	+	+	+	+
SP	++	+++	+++	+++	++	+++	+++	+++
XAN	+++	+++++	+++++	+++++	+++	+++++	+++++	+++++
XIC	+	+++	+++	+++	+	+++	+++	+++
BH	+	+	+	+	+	+	+	+
P I	+	+	+	+	+	+	+	+
P III	+	0	0	0	+	+	+	+
Y V	+	+	+	+	+	+	+	+
5'-AMP	+	+	+	+	+	+	+	+
GUAN-XA	+	+	+	+	+	+	+	+
IMP	+	+	+	+	+	+	+	+
UA	+++	+++	+++	+++	+++	+++	+++	+++

In all stocks, all three drosopterins were present in both sexes on day E1. As indicated in Tables 21-25, drosopterin I was found in small amounts, drosopterin II was present in only trace amounts, and drosopterin III was present in small-moderate amounts. The quantity of all drosopterins increased in both sexes in all stocks through day E10, after which time no further increase in the drosopterins was detectable. Visually estimated quantities of drosopterins, in order of decreasing magnitude, were drosopterin III > drosopterin I > drosopterin II (Tables 21-25).

An unknown blue-fluorescing compound (BH), an unknown yellow-fluorescing compound (Y V) which chromatographs directly above xanthopterin, and the unknown purple-fluorescing compound P I (which may be an artifact of chromatographic development) were present in trace amounts at all days sampled in both sexes and in all stocks. The original trace amounts of these compounds present on day E1 did not change with aging.

As indicated in Tables 21-25, the quantity of isoxanthopterin in heads of males and females did not change from that determined on day E1 and was consistently equal in males and females of a given stock. The quantity of isoxanthopterin in heads of ar 12 (Australia) was a small amount (Table 25) but was greater than that determined for any other stock (Tables 21-24). The quantity of isoxanthopterin in the Costa Rican stock (ar 11) was intermediate between the small amount present in ar 12 and the trace amount in ar 4 (Atlanta), ar 5 (Hawaii), and ar 6 (Yucatan).

Ar 11 differed qualitatively from all other stocks in having trace amounts of kynurenic acid and kynurenine throughout the aging period. By day E5, neither compound was present in any other stock.

Xanthopterin increased in all stocks from a small amount on day E1 to a moderate amount on day E5 (Tables 21-25). No further increase in xanthopterin was detectable after day E5.

A trace amount of P-6-COOH was present in both sexes of all stocks on day E1 (Tables 21-25). By day E10, the quantity of P-6-COOH in ar 4, ar 5, and ar 6 had increased to a small amount. In ar 11 and ar 12, the trace amount of P-6-COOH present on day E1 did not increase during the aging period.

Trace amounts of sepiapterin and isosepiapterin were detected in both males and females on day E1. The sepiapterin concentration increased in both sexes on day E5 to a small amount (Tables 21-25). Isosepiapterin increased to a trace-small amount on day E5. No further change in levels of these compounds was detectable during the remainder of the aging period. At no time was riboflavin found in heads of any of the stocks.

Small amounts of AHP-Biopterin were present in all stocks on day E1 with no detectable difference between stocks or between sexes. At day E5, AHP-Biopterin had increased to a small-moderate amount. By the third sampling day (E10), moderate amounts of AHP-Biopterin were present. At day E15, the quantity of AHP-Biopterin remained at moderate levels, as observed on day E10.

Xanthurenic acid was found in trace amounts in heads of both sexes in all stocks on day E1, as indicated in Tables 21-25. On day E5, the amount of xanthurenic acid had increased to a small amount. In ar 4, ar 6, and ar 12 there was no further change in xanthurenic acid during the remainder of the aging period, but in ar 5 and ar 11 levels of the compound increased to a small-moderate amount on day E15. At no time was a sexual difference noted in any stock.

The unknown purple-fluorescing compound P III was present in trace amounts on day E1 in all stocks. On day E5, P III had disappeared completely from male heads in all stocks except ar 11, in which it remained at a trace level. A trace amount of the compound was present in females of all stocks throughout the aging period.

Trace amounts of three UV-absorbing compounds (5'-AMP, IMP, and guanine-xanthine) were detectable in heads of both sexes of all stocks throughout the aging period. Uric acid was present in small amounts on day E1 in all stocks and remained at a small amount throughout the aging period in ar 5, ar 11, and ar 12. In ar 4 and ar 6, uric acid increased to a small-moderate amount on day E10, after which no further increase was noted.

Fluorescent and UV-Absorbing Compounds Isolated from Adult Bodies

Visually estimated quantities of fluorescent and UV-absorbing compounds isolated from bodies of males and females of the five stocks of *D. repleta* at days E1, E5, E10, and E15 are presented in Tables 26-30. As indicated, there are marked qualitative and quantitative differences

Table 26. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 4 Adult Male and Female Bodies at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	++	++	+++	++++	++	±	±	0
ISOSP	0	0	0	0	0	0	0	0
ISOX	++	+++	++++	+++++	++	+	±	0
KA	+	±	0	0	+	+	±	±
KYN	+	+	±	±	+	+	+	+
P-6-CH_3	+	+	+	+	+	++	+++	+++
P-6-COOH	0	±	+	+	0	0	0	0
RIB	+	++	+++	+++	+	++	+++	+++
SP	±	+	++	+++	±	0	0	0
XAN	±	+	++	++	±	+	+	+
XIC	+	±	0	0	+	±	0	0
BB	+	±	0	0	+	+	+	+
G I	+	+	++	++	+	+++	+++	++++

Table 26. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 4 Adult Male and Female Bodies at Days E1, E5, E10, and E15, Continued

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
P I	+	+	+	+	+	+	+	+
P III	+	+	+	+	+	+	+	+
P IV	+	0	0	0	+	0	0	0
WB	+	+	+	++	+	+	++	++
Y I	+	++	++	+++	+	++	++	+++
Y II	±	+	+	+	±	+	+	+
Y III	±	+	+	+	±	+	+	+
AD-A	0	++	+++	+++	0	+	++	+++
5'-AMP	++	++++	++++	+++++	++	++++	+++++	+++++
GUAN-XA	+	++	++	++	+	++	++	++
IMP	++	+	+	+	++	+	+	+
UA	++	+++	*	*	++	+++	+++++	+++++

*Masking by overlap with isoxanthopterin prevented estimate of quantity.

Table 27. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 5 Adult Male and Female Bodies at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	++	++	+++	++++	++	±	±	0
ISOSP	0	0	0	0	0	0	0	0
ISOX	++	+++	++++	+++++	++	+	±	0
KA	+	±	0	0	+	+	±	±
KYN	+	+	±	±	+	+	±	±
P-6-CH_3	+	+	+	+	+	+	++	+++
P-6-COOH	0	±	+	+	0	0	0	0
RIB	+	++	+++	+++	+	++	+++	+++
SP	±	+	++	+++	±	0	0	0
XAN	±	+	++	++	+	+	+	+
XIC	+	+	+	+	+	+	+	+
BB	±	+	+	+	±	+	+	+
G I	0	±	+	+	0	+	++	+++

Table 27. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 5 Adult Male and Female Bodies at Days E1, E5, E10, and E15, Continued

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
P I	+	+	+	+	+	+	+	+
P III	+	+	+	+	+	+	+	+
P IV	+	0	0	0	+	0	0	0
WB	+	+	+	+	+	++	++	+++
Y I	+	++	++	+++	+	++	+++	+++
Y II	±	+	+	+	±	+	+	+
Y III	±	+	+	+	±	+	+	+
AD-A	0	+	+++	+++	0	+	++	+++
5'-AMP	++	++++	++++	+++++	++	++++	+++++	+++++
GUAN-XA	+	++	++	++	+	++	++	++
IMP	++	+	+	+	++	+	+	+
UA	++	+++	*	*	++	+++	+++++	+++++

*Masking by overlap with isoxanthopterin prevented estimate of quantity.

Table 28. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 6 Adult Male and Female Bodies at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	++	++	+++	++++	++	±	±	0
ISOSP	0	0	0	0	0	0	0	0
ISOX	++	+++	++++	+++++	++	+	±	0
KA	+	±	0	0	+	+	±	±
KYN	+	+	±	±	+	+	+	+
P-6-CH_3	+	+	+	+	+	++	+++	+++
P-6-COOH	0	±	+	+	0	0	0	0
RIB	+	++	+++	+++	+	++	+++	+++
SP	±	+	++	+++	±	0	0	0
XAN	±	+	++	++	±	+	+	+
XIC	++	+	+	0	++	+	+	0
BB	+	+	±	0	+	+	+	+
G I	0	±	+	+	0	+	++	+++

Table 28. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 6 Adult Male and Female Bodies at Days E1, E5, E10, and E15, Continued

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
P I	+	+	+	+	+	+	+	+
P III	+	+	+	+	+	+	+	+
P IV	+	0	0	0	+	0	0	0
WB	+	+	+	++	+	+	++	++
Y I	+	++	++	+++	+	++	++	+++
Y II	±	+	+	+	±	+	+	+
Y III	±	+	+	+	±	+	+	+
AD-A	0	+	+++	+++	0	±	+++	+++
5'-AMP	++	++++	+++++	+++++	++	+++	+++++	+++++
GUAN-XA	+	++	++	++	+	++	++	++
IMP	++	+	+	+	++	+	+	+
UA	++	+++	*	*	++	+++	+++++	+++++

*Masking by overlap with isoxanthopterin prevented estimate of quantity.

Table 29. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 11 Adult Male and Female Bodies at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	++	+++	++++	+++++	++	±	±	0
ISOSP	0	±	+	++	0	0	0	0
ISOX	+++	++++	+++++	++++++	+++	++	±	0
KA	+	0	0	0	+	±	±	±
KYN	++	++	+	+	+	+	+	+
P-6-CH_3	+	+	++	+++	+	++	+++	++++
P-6-COOH	0	±	+	++	0	0	0	0
RIB	±	+	++	+++	±	+	++	+++
SP	+	++	+++	++++	+	±	0	0
XAN	+	++	++	+++	+	+	+	+
XIC	±	0	0	0	±	0	0	0
BB	+	0	0	0	+	+	+	+
G I	0	0	0	0	0	0	0	0

Table 29. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 11 Adult Male and Female Bodies at Days E1, E5, E10, and E15, Continued

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
P I	+	+	+	+	+	+	+	+
P III	+	+	+	+	+	+	+	+
P IV	+	0	0	0	+	0	0	0
WB	+	++	++	++	+	++	++	+++
Y I	+	++	++	+++	+	++	++	+++
Y II	±	+	+	+	±	+	+	++
Y III	±	+	+	+	±	+	+	++
AD-A	0	++	+++	+++	0	++	+++	+++
5'-AMP	++	+++	++++	++++	++	++++	+++++	+++++
GUAN-XA	+	+	++	++	+	++	+++	+++
IMP	+	++	++	++	+	++	++	++
UA	+++	*	*	*	+++	+++	++++	+++++

*Masking by overlap with isoxanthopterin prevented estimate of quantity.

Table 30. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 12 Adult Male and Female Bodies at Days E1, E5, E10, and E15

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
AHP-BIO	+++	++++	+++++	++++++	+++	++	±	0
ISOSP	±	+	++	+++	±	0	0	0
ISOX	++++	+++++	++++++	+++++++	++++	++	±	0
KA	+	+	+	+	+	+	+	+
KYN	++	+	+	+	++	+	+	+
P-6-CH_3	±	±	±	±	±	+	++	+++
P-6-COOH	±	+	++	++	+	0	0	0
RIB	+	++	+++	++++	+	++	+++	++++
SP	+	+++	++++	+++++	+	0	0	0
XAN	+	++	+++	++++	+	+	+	+
XIC	+++	+	±	±	+++	+	±	±
BB	0	0	0	0	0	0	0	0
G I	0	0	0	0	0	0	0	0

Table 30. Visually Estimated Quantities of Fluorescent and UV-Absorbing Compounds Isolated from ar 12 Adult Male and Female Bodies at Days E1, E5, E10, and E15, Continued

Compound	Males				Females			
	E1	E5	E10	E15	E1	E5	E10	E15
P I	+	+	+	+	+	+	+	+
P III	+	+	+	+	+	+	+	+
P IV	+	0	0	0	+	0	0	0
WB	+	+	+	+	+	+	++	+++
Y I	+	+	++	+++	+	++	++	+++
Y II	±	+	+	+	±	+	++	++
Y III	±	+	+	+	±	+	++	++
AD-A	0	++	+++	+++	0	++	+++	+++
5'-AMP	+++	+++++	+++++	+++++	+++	+++++	++++++	++++++
GUAN-XA	±	±	++	+++	±	±	++	+++
IMP	++	++	++	++	++	++	++	++
UA	+++	*	*	*	+++	+++	+++++	+++++

*Masking by overlap with isoxanthopterin prevented estimate of quantity.

in fluorescent and UV-absorbing compounds between males and females of each stock, as well as interstock differences among males and among females.

On day E1, patterns of fluorescent and UV-absorbing compounds in bodies of males and females of a given stock were virtually indistinguishable both qualitatively and quantitatively (Tables 26-30). However, differences between stocks were apparent on day E1. The amount of isoxanthopterin in ar 12 (small-moderate) exceeded that found in ar 11 (small), which was greater than in stocks ar 4, ar 5, and ar 6 (trace-small). A trace amount of the unknown green-fluorescing compound G I was present on day E1 in ar 4 (Table 26), while this compound was absent in ar 5 (Table 27) and ar 6 (Table 28) until day E5 and never appeared in ar 11 (Table 29) or ar 12 (Table 30).

By day E5, chromatograms of male and female bodies were readily distinguishable in all stocks. Amounts of isoxanthopterin, xanthopterin, sepiapterin, AHP-Biopterin, and P-6-COOH in E5 male bodies far exceeded the amounts present in females. In males of all stocks during aging (Tables 26-30) there was a consistent and obvious increase in amounts of isoxanthopterin, xanthopterin, P-6-COOH, sepiapterin, isosepiapterin, and AHP-Biopterin, while females of all stocks showed either a decrease or little change in quantities of the same compounds.

The increase in amounts of isoxanthopterin, xanthopterin, P-6-COOH, sepiapterin, isosepiapterin, riboflavin, and AHP-Biopterin in male bodies during the 15 day aging period was consistently greater in ar 11 and ar 12 than in ar 4, ar 5, and ar 6. While these compounds

were present at higher levels in ar 11 and ar 12, the unknown green-fluorescing compound G I, which increased in ar 4, ar 5, and ar 6 males during the aging period, was absent in ar 11 and ar 12 males.

Fluorimetric Quantitation of Isoxanthopterin Isolated from Adult Bodies

The mean concentrations of isoxanthopterin (± standard error of the mean) isolated from adult male and female decapitated bodies of each stock of D. repleta at days E1, E5, E10, and E15 are presented in Table 31. The isoxanthopterin concentrations determined for individual samples of males and females of each stock on each sampling day are listed in Appendix B, Tables 11-12.

Results of the small-sample Student t test evaluation of male-to-female comparisons of mean isoxanthopterin concentrations in each stock on days E1, E5, E10, and E15 are also presented in Table 31. On day E1, the Student t evaluation revealed no significant difference between mean isoxanthopterin concentrations in males and females of stocks ar 4, ar 5, or ar 6. However, the Student t evaluation did reveal significant differences between males and females in stocks ar 11 and ar 12 ($p < 0.001$). At days E5, E10, and E15, the differences in mean isoxanthopterin concentration in males and females were significant in all stocks ($p < 0.001$).

The obvious increase in isoxanthopterin concentration with time after eclosion in males of all D. repleta stocks and the obvious decrease in isoxanthopterin concentration with time after eclosion in females of all stocks are graphically represented in Figure 4 and

Table 31. Mean Concentrations of Isoxanthopterin[1] Isolated from Adult Male and Female Decapitated Bodies of Each Stock at Days E1, E5, E10, and E15

Stock and Sex	E1	E5	E10	E15
ar 4 Male	3.1 ± 0.15 (N = 10)	66.4 ± 5.46 (N = 10)	123.0 ± 13.33 (N = 10)	232.0 ± 12.07 (N = 8)
ar 4 Female	2.9 ± 0.18 (N = 10)	1.5 ± 0.38 (N = 10)	1.2 ± 0.35 (N = 10)	1.0 ± 0.16 (N = 10)
Statistics[2]	$p < 0.5$	$p < 0.001$	$p < 0.001$	$p < 0.001$
ar 5 Male	3.6 ± 0.39 (N = 8)	87.1 ± 9.22 (N = 10)	215.9 ± 14.23 (N = 10)	265.0 ± 14.83 (N = 10)
ar 5 Female	3.1 ± 0.20 (N = 9)	1.2 ± 0.14 (N = 10)	1.3 ± 0.12 (N = 9)	1.3 ± 0.31 (N = 10)
Statistics[2]	$p < 0.4$	$p < 0.001$	$p < 0.001$	$p < 0.001$
ar 6 Male	4.8 ± 0.58 (N = 9)	55.5 ± 7.50 (N = 10)	152.1 ± 20.41 (N = 10)	261.4 ± 9.64 (N = 10)
ar 6 Female	4.0 ± 0.37 (N = 10)	1.6 ± 0.12 (N = 10)	1.5 ± 0.12 (N = 10)	1.2 ± 0.10 (N = 10)
Statistics[2]	$p < 0.4$	$p < 0.001$	$p < 0.001$	$p < 0.001$

[1]Values are expressed as (Mean ± Standard Error) x 10^3 µg/mg wet weight of sample.

[2]Student *t* evaluation of male vs. female differences in mean isoxanthopterin concentration.

Table 31. Mean Concentrations of Isoxanthopterin[1] Isolated from Adult Male and Female Decapitated Bodies of Each Stock at Days E1, E5, E10, and E15, Continued

Stock and Sex	E1	E5	E10	E15
ar 11 Male	12.1 ± 0.66 (N = 9)	152.3 ± 4.12 (N = 10)	261.8 ± 8.38 (N = 10)	347.2 ± 16.75 (N = 10)
ar 11 Female	7.6 ± 0.58 (N = 10)	3.3 ± 0.75 (N = 10)	1.6 ± 0.11 (N = 10)	1.4 ± 0.15 (N = 10)
Statistics[2]	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
ar 12 Male	67.9 ± 4.42 (N = 9)	255.6 ± 9.80 (N = 10)	450.3 ± 9.59 (N = 10)	495.8 ± 17.14 (N = 10)
ar 12 Female	7.9 ± 0.54 (N = 10)	1.6 ± 0.07 (N = 10)	1.2 ± 0.05 (N = 10)	1.1 ± 0.08 (N = 10)
Statistics[2]	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

[1]Values are expressed as (Mean ± Standard Error) x 10^3 µg/mg wet weight of sample.

[2]Student t evaluation of male vs. female differences in mean isoxanthopterin concentration.

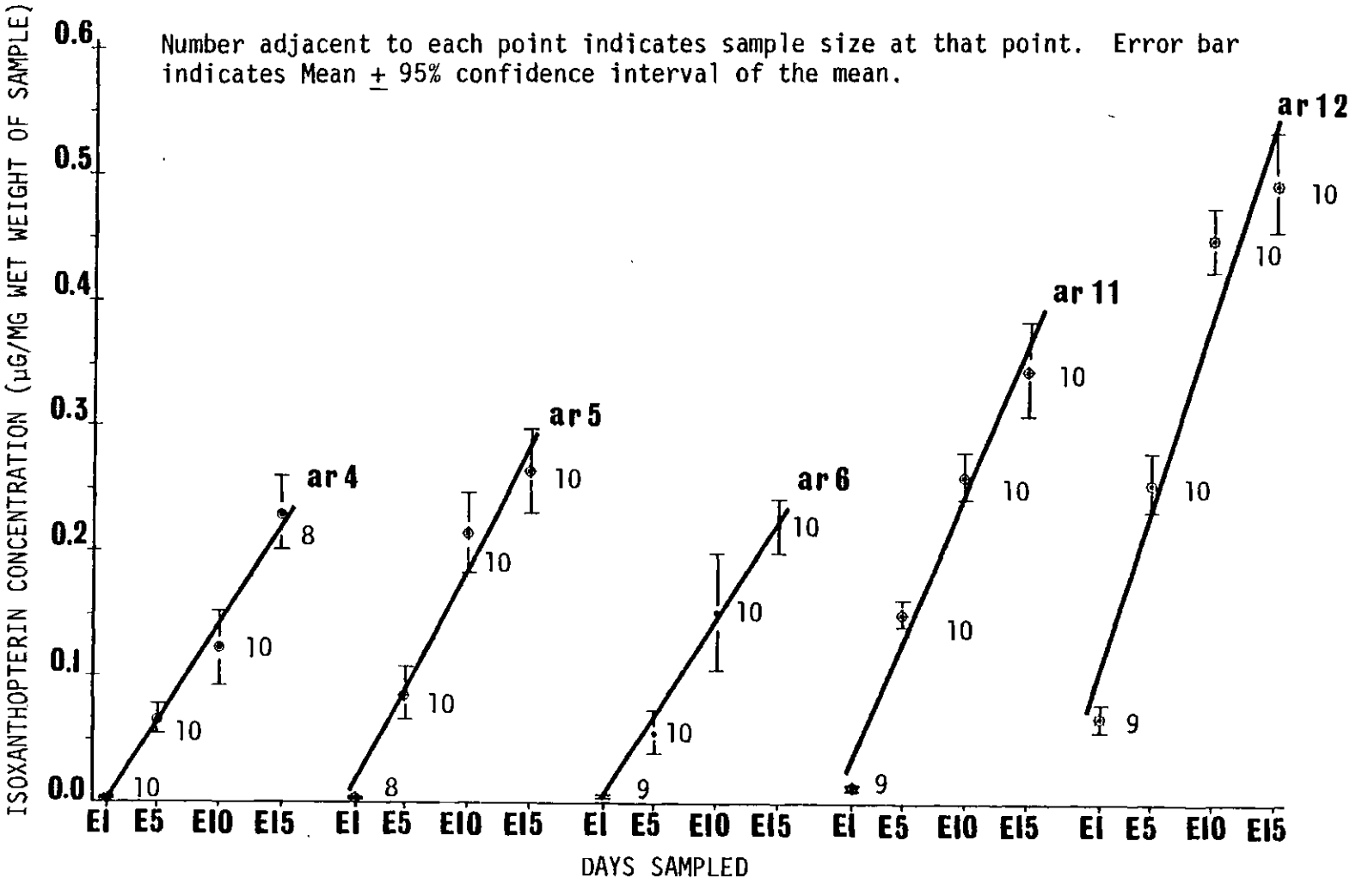

Figure 4. Isoxanthopterin Concentration vs. Time After Eclosion in Males

Figure 5, respectively. Attempts to transform the female data for each stock so as to permit examination by linear regression were unsuccessful. Figure 5 graphically represents mean concentrations of isoxanthopterin (± the 95% confidence intervals of the mean) in females of each stock on each day sampled. Superimposed on the mean values for females of each stock are lines which were fitted with a French curve to indicate the general trend of decrease in isoxanthopterin concentration with time after eclosion.

The mean isoxanthopterin concentrations (± the 95% confidence intervals of the mean) for each day sampled in males of each stock are plotted in Figure 4. Change in isoxanthopterin concentration with time after eclosion in males of each stock was examined by determination of the equation of the least squares linear regression line for each stock. Table 32 contains the equations determined for least squares linear regression lines in each stock, as well as the 95% confidence intervals of the mean isoxanthopterin concentration for each line. Also included in Table 32 is the 95% confidence interval for the slope of each of the regression lines. Figure 6 is a graphic representation of the equation of the least squares linear regression lines determined for males of each stock, providing a simplified visual comparison of the changes in isoxanthopterin concentration with time after eclosion.

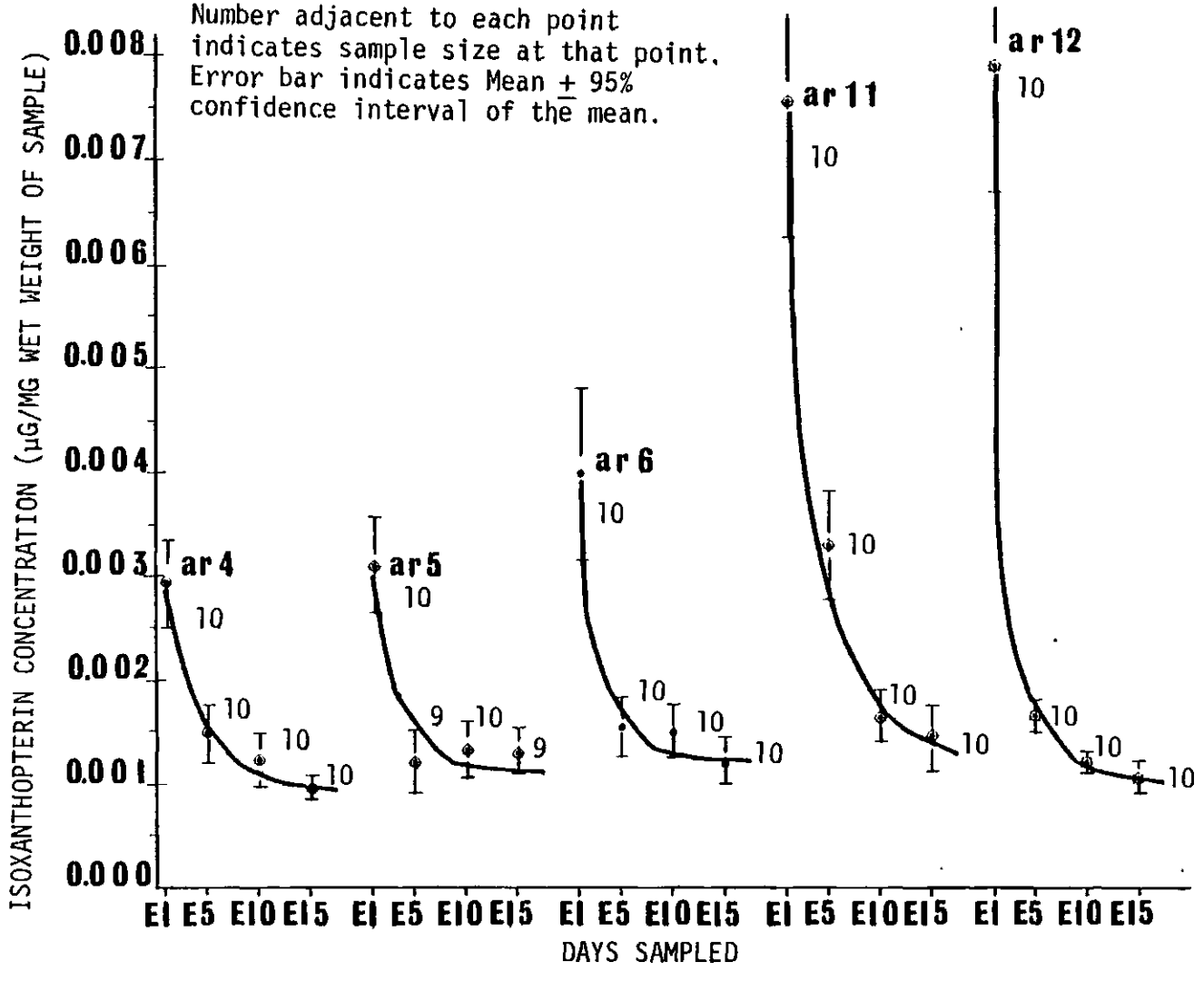

Figure 5. Isoxanthopterin Concentration vs. Time After Eclosion in Females

Table 32. Equations of the Least Squares Linear Regression Lines Determined for Concentrations of Isoxanthopterin[1] Isolated from Adult Male Decapitated Bodies at Days E1, E5, E10, and E15

Stock	$y = \bar{y} + b\ (x - \bar{x})$[2,3,4]	95% Confidence Interval for $\bar{y}$ at $\bar{x}$	95% Confidence Interval for Slope of Regression Line[5]	N[6]
ar 4	y = 0.099 + 0.016 (x - 7.37)	y = 0.090 to 0.109	B = 0.014 to 0.018	38
ar 5	y = 0.150 + 0.019 (x - 8.11)	y = 0.137 to 0.164	B = 0.017 to 0.022	38
ar 6	y = 0.111 + 0.016 (x - 7.92)	y = 0.099 to 0.123	B = 0.014 to 0.018	39
ar 11	y = 0.198 + 0.023 (x - 7.92)	y = 0.186 to 0.210	B = 0.021 to 0.026	39
ar 12	y = 0.324 + 0.031 (x - 7.92)	y = 0.305 to 0.343	B = 0.027 to 0.035	39

[1]Concentration of isoxanthopterin expressed as µg/mg wet weight of sample.

[2]$\bar{y}$ = General mean concentration of isoxanthopterin, from linear regression line.

[3]b = Slope of regression line, expressed as µg/mg/day.

[4]$\bar{x}$ = General mean sampling age, from linear regression line.

[5]B is expressed in µg/mg/day. (Since confidence intervals do not contain zero, there is a real and positive correlation between isoxanthopterin concentration and increasing age.)

[6]N = Total sample size (Number of samples determined for days E1, E5, E10, and E15).

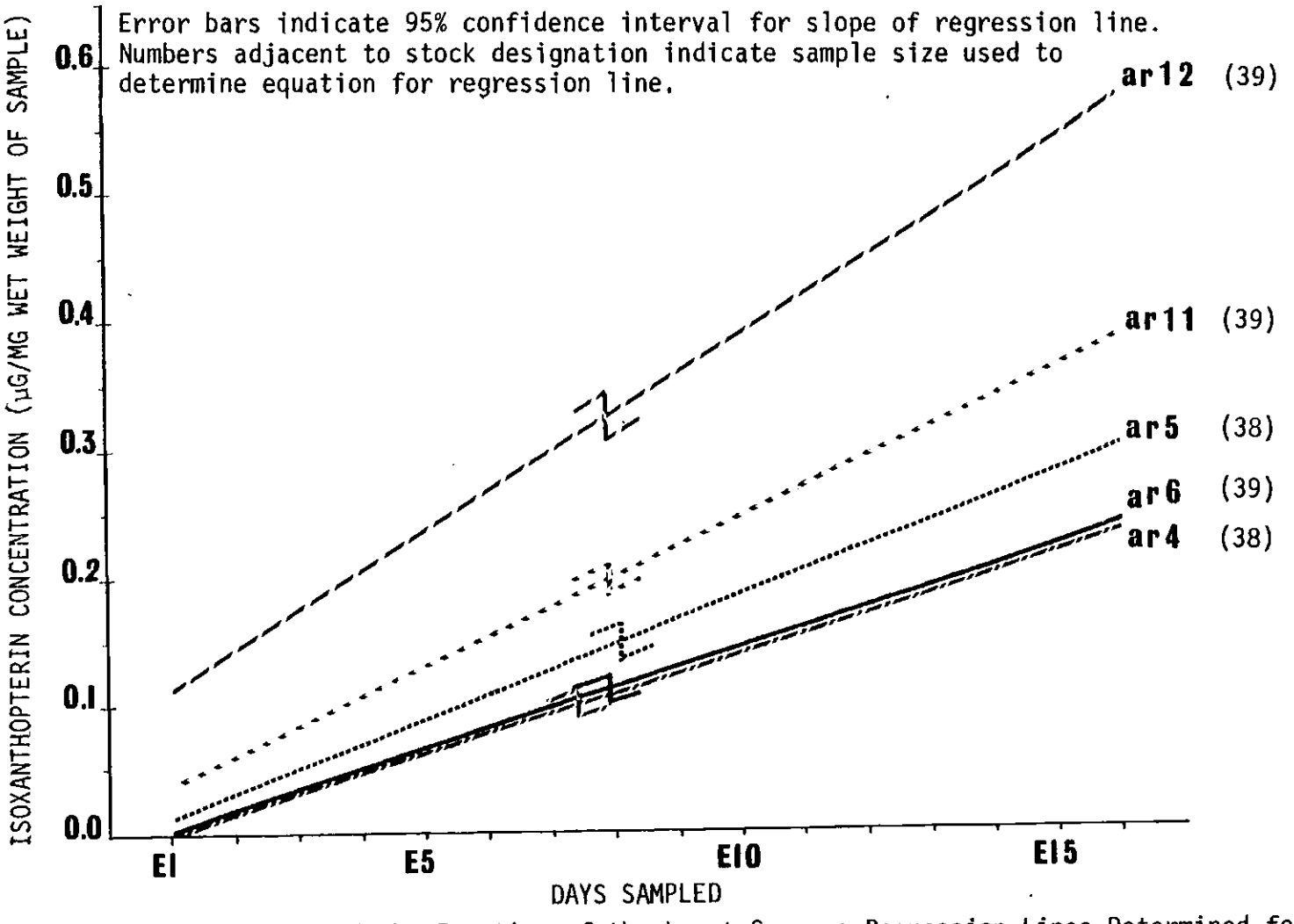

Figure 6. Graphic Representation of the Equation of the Least Squares Regression Lines Determined for Relationship between Isoxanthopterin Concentration and Time after Eclosion in Males

CHAPTER IV

DISCUSSION

Cross-Fertility of ar 1000 with ar 4, ar 5, ar 6, ar 11, and ar 12

The species D. repleta is cosmopolitan in distribution (Patterson and Stone, 1952) and in nature tends to form larger populations in the autumn than in any other season (Spencer, 1950). D. repleta wild type stocks have been collected in the autumn months in the general Atlanta area at three locations (Colley, 1967; Jocoy, unpublished collection data), and laboratory cultures of the species had not been maintained in the area prior to their original capture.

There have been two studies examining reproductive isolation among wild type stocks of D. repleta which were collected in geographically diverse locations (Wharton, 1941, 1942; Humphrey, 1974; Colley and Humphrey, manuscript submitted for publication). Both studies used stocks of D. repleta which had been in laboratory culture for extended periods of time. Humphrey (1974) investigated reproductive isolation among a total of six stocks, five of which were included in every phase of the present study.

One of the conclusions drawn from Humphrey's study was that geographical isolation of the stocks from Australia (ar 12) and Costa Rica (ar 11) had been accompanied by reproductive isolation, while stocks from Georgia (ar 4, Atlanta), Connecticut (ar 13), Mexico (ar 6, Yucatan), and the Hawaiian island of Oahu (ar 5) formed a freely

interbreeding group. When a wild type stock of D. repleta (ar 1000, Atlanta) was collected in the Fall of 1974 (and again in the Fall of 1975), it was convenient to perform a limited number of pair and mass matings for the purpose of determining in a cursory manner whether the degree of success in interstock crosses was influenced by sustained maintenance in laboratory culture.

Crosses involving males of ar 1000 (Atlanta, new stock) with females of ar 4 (Atlanta), ar 5 (Hawaii), and ar 6 (Yucatan), as well as the reciprocal crosses, were successful in both pair and mass matings (Table 5). Pair and mass matings of ar 1000 males with females of ar 11 (Costa Rica) and ar 12 (Australia) were unsuccessful, as were the reciprocal crosses.

The results of this limited study indicate no qualitative differences between ar 4 and ar 1000 in success of matings with other stocks and provide evidence that fixation of genetic differences did not account for the lack of success in matings between Atlanta and Costa Rica or Australia observed by Humphrey (1974). The results also are in agreement with Humphrey's report of reproductive isolation of ar 11 and ar 12 from the freely interbreeding group including ar 4, ar 5, and ar 6 (ar 13 not tested), as well as ar 1000.

Timing of Developmental Stages and Morphological Characteristics

An initial assumption in the daily examination of chromatographic patterns of fluorescent and UV-absorbing compounds in the five wild type stocks of D. repleta during development was a similarity among stocks in

timing of developmental stages and in total time required to complete the life cycle. This assumption was found to be incorrect (Table 6). Results of this study of timing of developmental stages during the life cycle of each stock allows a rather clear distinction to be made between groupings of these stocks.

The timing of embryogenesis from fertilized egg to first instar larva permitted three distinct groupings of the five stocks (Table 6). One group includes ar 11 and ar 12, in which the period of embryogenesis was approximately 24 hours. The period of embryogenesis in ar 5 and ar 6 encompassed approximately 36 hours, while ar 4 required 2-3 days to complete embryogenesis. The mean interval of embryogenesis reported for Atlanta stock ar 1, from which ar 4 was derived, encompassed 2.0 ± 0.1 days (Colley, 1967). While embryogenesis required 1/2 to 1 1/2 days longer in ar 4, after the first larval instar all developmental stages encompassed the same amount of time in ar 4, ar 5, and ar 6. Thus, by the onset of the second instar larval stage, ar 4, ar 5, and ar 6 were temporally grouped and remained so throughout the remainder of development.

The morphological characteristics of developmental stages of all five stocks, as described in Results, indicated a consistent pattern of morphological change in all stocks which may be considered characteristic of the species. Except for the distinction of ar 12 in having an off-white larval body color instead of a yellowish body color and in its failure to develop brownish-red coloration of the anterior spiracles prior to pupation, all stocks were essentially identical in developmental

morphology.

Several morphological differences in adults, including differences in pigmentation of eyes and bodies, phenotypically distinguished both Australian and Costa Rican stocks from each other and from the group consisting of the other three stocks. For example, the mature adult D. repleta is classically described as having sepia eyes and dark brown bands on the dorsal surface of the abdomen (Patterson, 1943). The Atlanta, Yucatan, and Hawaiian stocks conform to the classic description of the species in pigmentation, as well as in reproductive morphology (Humphrey, 1974), while Australian and Costa Rican stocks differ in morphology of spermathecae and ventral receptacles (Humphrey, 1974) and in pigmentation. The eyes of mature Australian flies are brick-red, and the testes are brighter yellow than in any other stock. The Costa Rican stock has bright red eyes which do not darken with age, and the dorsal abdominal bands are paler brown, producing the effect of a distinctly lighter body color than in other stocks.

Discussion of Chromatographic Methods

In comparing results of this study with those from previous related studies (Hadorn and Mitchell, 1951; Throckmorton, 1962; Colley, 1967; Howell, 1969), certain pertinent differences in sample size, method of preparing samples for chromatography, method of chromatographic development, and solvents used for development should be noted. Obviously, differences in size of samples could be expected to influence differences in qualitative results. Compounds which may be present in

neglibible amounts in small samples, so as to be undetectable by visual inspection of chromatograms with UV light, would obviously be more easily detected when the sample size is increased. At the same time, the absence of specific compounds might, in fact, directly reflect differences in biosynthetic pathways or metabolic activities of the organisms sampled.

The method of preparation of pteridine-containing samples has been examined and found to influence the numbers of compounds isolated (Hadorn and Mitchell, 1951; Ziegler, 1961; Rembold, 1970). As discussed in the Methods section of the present study, boiling of larval samples in direct contact with water was found to lead to extraction of a number of fluorescent compounds. On chromatograms of these samples, the disadvantages of loss of quantities and failure to localize certain compounds were evident. Loss of material was verified by comparison of chromatograms prepared from unboiled larval samples with those prepared by boiling in water and by dry-boiling. The dry-boiling technique was deemed most satisfactory in that it minimized both loss of material by extraction and the trailing of compounds on chromatograms of unboiled samples due to interference by proteins.

Ziegler (1961) and Rembold (1970), among others, have reported that heat denaturation of pteridine-containing samples in the presence of oxygen can lead to the production of simple pteridines, as well as increased amounts of other degradation products. The simple pteridines produced are degradation products of the dihydro- and tetrahydropteridines. However, similar oxidative degradation products have been

reported for chromatograms of unboiled samples (Ziegler and Harmsen, 1969; Blakley, 1969).

Qualitative and quantitative studies of pteridines in Drosophila by paper chromatography have utilized both ascending and descending development. A number of different solvent mixtures have been used in these studies. In the present study, ascending two-dimensional paper chromatography was the preferred method of development. The major advantage of two-dimensional separation is in the isolation of overlapping compounds which have very similar Rf values in a given solvent system. The only two pteridines not resolvable by this procedure were AHP and biopterin. Separation of these two compounds required pre-equilibration of the chromatogram prior to development, which was a part of the descending chromatographic procedure involving two single-dimensional developments in two different solvent systems employed in related studies by Throckmorton (1962), Colley (1967), and Howell (1969).

Fluorescent and UV-Absorbing Compounds in Development

In view of the absence in the literature of a daily sequential developmental analysis of pteridine patterns and UV-absorbing compounds in the species D. repleta, the present characterization and comparative analysis of changes in patterns of fluorescent and UV-absorbing compounds during development and aging in five stocks of D. repleta from different geographical regions were undertaken. This study is an attempt to provide a biochemical characterization of the species as well as to evaluate the extent of intraspecific variation among stocks.

The timing of appearance and quantitative fluctuations in fluorescent and UV-absorbing compounds appears to be generally similar in the five stocks at any given stage of development, although the specific day of appearance or disappearance of a given compound may vary among stocks due to differences in timing of developmental stages (Table 6).

In each of the five stocks, isoxanthopterin, xanthopterin, riboflavin, kynurenic acid, kynurenine, an unknown purple-fluorescing compound (P III), ATP-GTP, and 5'-AMP were present in eggs and embryos and throughout the remainder of development. In addition to the compounds listed above, AHP-Biopterin, P-6-CH_3, P I (the unknown purple-fluorescing compound which may be an artifact of the chromatographic procedure), an unknown burnt-yellow fluorescent compound (BY I), uric acid, and guanine-xanthine were present for the first time in all stocks during the first instar larval stage and throughout the rest of development.

Compounds appearing for the first time in all stocks at the second instar larval stage and present throughout the rest of development were xanthurenic acid and hypoxanthine. No additional compounds were added to those already present during previous developmental stages until late in the pupal stage when pupae have begun to take on adult coloration. At this stage, the eye pigments drosopterin I and drosopterin III were present in all stocks. The eye pigment drosopterin II, which was the last compound to be added to those already

present during previous developmental stages, did not appear until the final 24 hours of pupation.

In addition to the eight compounds already indicated as present throughout development, eggs of the five stocks of D. repleta were found to have an unknown yellow-fluorescing compound (Y I), an unknown green-fluorescing compound (G II), and inosine monophosphate. Slight trace levels of uric acid were present in eggs of ar 4, ar 5, and ar 6. Hadorn and Mitchell (1951), in a study of fluorescent compounds present during development in D. melanogaster, reported the presence of only one compound in eggs, a lemon-colored fluorescent compound (FL2). The chromatographic characteristics and fluorescent color of FL2 are similar to those reported here for compound Y I, and they may be the same compound. Hadorn and Mitchell reported that FL2 was present throughout development, as well as in adults, while in the five stocks of D. repleta, Y I disappeared and reappeared a number of times during development.

Colley (1967), in a paper chromatographic analysis of the pteridines in developmental stages and in selected adult ages of an Atlanta wild type stock of D. repleta (ar 1), found traces of isoxanthopterin, a xanthopterin-like compound, a riboflavin-like compound, and a kynurenine-like compound in eggs and embryos as well as throughout development. She did not observe sexual differences in pteridine patterns of third instar larvae of D. repleta, in contrast to the findings of Hadorn and Mitchell (1951) for D. melanogaster. In contrast to the results reported here, Colley did not find any additional

compounds appearing during development until the third instar larval stage. In addition to the pteridines which appeared at earlier developmental stages, she reported in third instar larvae traces of sepiapterin and unknown yellow- and green-fluorescing compounds and an increase in the kynurenine-like compound and riboflavin.

Throckmorton (1962) reported results of an extensive study of the distribution of fluorescent compounds in the genus Drosophila. Included in his study was a stock of D. repleta from the British West Indies. The only developmental stage studied by Throckmorton was late third instar larva. He reported the presence of unknown green- and yellow-fluorescing compounds, xanthopterin, riboflavin, uric acid, and a kynurenine-like compound with blue-violet fluorescence. The xanthopterin-like compound reported by Colley (1967) and Throckmorton (1962) is not the same compound as is reported in this study. In fact, it would appear that the compound previously described as xanthopterin-like may have been kynurenic acid since both compounds have a green fluorescence and similar chromatographic characteristics and no compound identified as kynurenic acid was reported in the previous studies.

At least two explanations related to differences in procedures of sample preparation and chromatography are possible for the larger number of compounds isolated during development in the present study than in studies of Throckmorton (1962) and Colley (1967). First, they used a smaller sample size. Obviously, as the size of the sample is increased, compounds which may be present in limited quantities would

be more likely to be detected. Second, the method of sample preparation was different (boiling in water by Throckmorton and by Colley versus dry-boiling in this study), as was the method of chromatography (one-dimensional descending chromatography using two solvents in separate developments versus two-dimensional ascending chromatography in this study), which may have provided for finer resolution of certain trace-level compounds that were masked by overlap with more abundant compounds after one-dimensional development.

Active embryonic synthesis of pteridines has been demonstrated during embryogenesis in the milkweed bug Oncopeltus (Forrest, 1970), but has not been clearly demonstrated in Drosophila. The presence of pteridines in the eggs of D. repleta does not of necessity indicate that they are products of a metabolically active synthetic pathway within the embryo itself. Pteridines present at this stage of development may be entirely products of metabolism of the maternal tissues and fluids to which eggs were exposed prior to oviposition (Ziegler and Harmsen, 1969; Nickla, 1973).

In contrast to the results of Colley's (1967) study of D. repleta, there was a distinctive increase in the number of fluorescent and UV-absorbing compounds in first instar larvae of all stocks when compared to eggs (Tables 15-20). There was also a general increase in the amounts of all compounds which were present in eggs and embryos during the first instar larval stage. The appearance of AHP-Biopterin during the first larval instar, as well as the general increase in amounts of

pteridines and other fluorescent compounds, indicates active synthesis during this stage.

The appearance of the seven yellow and white fluorescent compounds (BY I, BY II, Y IV, W I, W II, W III, and WB) during the first larval instar in all stocks appears to be related to larval metabolism. All of these compounds except for BY I and WB appear to be metabolically associated exclusively with larval stages of development, as does the compound G II, since by the late third instar larval stage they have either completely disappeared or have had their quantitative levels reduced to trace amounts. The unknown UV-absorbing compound UV I present during the first instar larval stage may be an intermediate in or other wise metabolically associated with the synthesis of the unknown yellow and white fluorescent compounds since it disappears during the early third instar stage, and it is during the second and third instars that the unknown yellow and white compounds consistently and progressively decrease.

During the first instar larval stage a quantitative difference between stocks is observed in the amounts of isoxanthopterin and riboflavin. Ar 12 has a greater amount of isoxanthopterin and a smaller amount of riboflavin than do stocks ar 4, ar 5, and ar 6. Stock ar 11 is intermediate between ar 12 and the group of stocks ar 4, ar 5, and ar 6 in the quantities of these compounds. Ar 11 and ar 12 continue to have greater amounts of isoxanthopterin and smaller amounts of riboflavin throughout larval development.

The low levels of pteridines relative to amount of riboflavin during the second larval instar (Tables 15-17) and the reversal of this relationship in all stocks at later stages of larval and pupal development support the inverse relationship between pteridine synthesis and riboflavin accumulation proposed in studies with D. melanogaster (Ziegler, 1961; Nickla, 1972, 1973). The appearance of the unknown purple-fluorescing compound P II during the first instar and its disappearance by pupation indicates that it also is associated with larval development.

Since both Colley (1967) and Throckmorton (1962) used water-boiled samples of third instar larvae, it is difficult to make qualitative or quantitative comparisons with their results. Yet, in agreement with results of Throckmorton and in contrast to those of Colley, no sepiapterin or isosepiapterin were found in third instar larvae. This discrepancy may be explained by the fact that in third instar larvae the riboflavin-like compound reacted to exposure to ammonia vapors in such a way as to permit its being viewed as two compounds. Since these compounds are not chromatographically separable, the discrepancy may simply reflect different interpretations by different investigators.

During the period encompassing the third larval instar there is a general quantitative decrease in or stabilization of all compounds, except for increases in isoxanthopterin and kynurenine. The number and concentration of the UV-absorbing compounds decreased in all stocks during the late third larval instar.

The patterns of UV-absorbing compounds present throughout development in D. repleta support the presence of an active metabolic pathway of purine conversion to uric acid (Gilmour, 1965). The presence of the UV-absorbing compounds and their fluctuations during development are in agreement with reports that in Drosophila xanthine oxidase activity is higher in the larval stage than in the pupal stage (Ursprung and Hadorn, 1961; Munz, 1964). Based on the decrease in the purine metabolites and uric acid in late third instar larvae, it could be speculated that the xanthine oxidase activity in D. repleta is higher during this phase of development. The decrease in UV-absorbing compounds could also reflect a simple attempt to remove metabolically unnecessary or harmful concentrations of nitrogenous materials prior to pupation, from which time no additional exchange of materials between the organism and its environment will be possible until eclosion. The decrease in purines prior to pupation could also reflect the operation of an allosteric control mechanism regulating activity of xanthine oxidase, which functions in conversion of 2-amino-4-hydroxypteridine (AHP) to isoxanthopterin in D. melanogaster (Forrest et al, 1956; Hadorn and Graf, 1958; Glassman and Mitchell, 1959; Okada and Goto, 1965).

In agreement with previously reported studies of D. melanogaster (e.g., Hadorn and Mitchell, 1951; Fan et al, 1976) and D. repleta (Colley, 1967), major qualitative and quantitative changes in fluorescent compounds are most marked during the pupal stage of development. In the early pupa before onset of adult coloration, Colley found traces of drosopterins I and III and small amounts of AHP, biopterin, and an

unknown blue-fluorescing compound, none of these compounds having appeared earlier in development. Sepiapterin, which had been present in third instar larvae, disappeared, as did the unknown green-fluorescing compound, and the quantity of riboflavin decreased. In pre-eclosion pupae after onset of adult coloration, drosopterin II appeared for the first time and the amount of riboflavin increased again, as did the unknown yellow compound. All other compounds remained at previous levels.

In D. melanogaster, ommochromes begin to appear at 53-55 hours after the beginning of pupation and the drosopterins begin to appear in eyes at 71 hours after the onset of pupation (Danneel, 1941). In all five stocks of D. repleta, ommochromes did not appear until 60-72 hours after the onset of pupation (observation of reddish-brown coloration of pupal eyes, Table 6) and the drosopterins appeared 96-108 hours after pupation had begun (Tables 6 and 15-17). The quantitative increase observed in the ommochrome precursors and metabolites during pupation in D. repleta is similar to that reported for D. melanogaster (Hadorn and Ziegler, 1958; Ryall and Howells, 1974; Sullivan et al, 1973).

Quantitative changes in xanthurenic acid, kynurenic acid, and kynurenine during pupal development allow subdivision of the five stocks of D. repleta into three groups. The pattern of quantitative changes in these three compounds during pupation is identical in ar 4, ar 5, and ar 6, in which kynurenine increases, while xanthurenic acid and kynurenic acid remain at trace levels. Ar 11 is distinguished from all other stocks in that kynurenine does not increase, while increases in both kynurenic acid and xanthurenic acid are observed on the day that

the eyes of pupae take on a detectable reddish-brown color. Kynurenine increases on the day that ommochromes become visible in ar 12, as is found in ar 4, ar 5, and ar 6, but in ar 12 during the last two days of pupal development levels of kynurenine, which remain elevated in ar 4, ar 5, and ar 6, decrease to a trace.

The quantitative differences among stocks in the ommochrome synthesis intermediates xanthurenic acid, kynurenic acid, and kynurenine during the last half of pupal development are consistent with differences in the final phenotypic expression of adult eye color. What is not explained by the data presented here is the fact that all stocks have a scarlet eye color on the day of eclosion. While no definite conclusions can be drawn, certain suppositions and speculations are possible. The differences in adult eye color in the different groupings of stocks are associated with increased levels of ommochrome precursors in ar 4, ar 5, and ar 6 during the last days of development, and darkening of the eye color with age may reflect a higher rate of ommochrome synthesis in these stocks. A possible explanation for the gradual change in eye color from scarlet to sepia in these stocks may lie in the chemical state of the ommochromes during aging. The eyes of all stocks at eclosion have a color similar to that reported for the reduced form of xanthommatin (Ziegler, 1961). This particular ommochrome is generally found in nature in its oxidized form, in which it has a yellowish-brown color. If, during the aging period, the oxidation state of eye tissue changes, the conversion of the reduced ommochrome to the oxidized form could account for or contribute to the darkening of

adult eyes (cf. Rembold, 1970).

Major qualitative and quantitative differences in the patterns of pteridines and pteridine-related compounds during development were most marked in the pupal stage (Tables 15-17). The timing of appearance of the drosopterins (Tables 15-17) was similar in all stocks in that these compounds did not appear until the day after the appearance of the reddish-brown color of the pupal eyes (approximately 48 hours prior to eclosion). Drosopterins I and III appeared approximately 24 hours earlier than drosopterin II and were always present in greater quantities. Levels of the drosopterins appear to be slightly lower in ar 11 and ar 12 than in the other stocks. These observations are consistent with the time of appearance of drosopterins reported by Colley (1967). The relative time and sequence of appearance of the drosopterins in D. repleta and the increase in these compounds during the period immediately prior to and following eclosion are also essentially consistent with results of studies of D. melanogaster, although D. repleta differs from D. melanogaster in that drosopterin synthesis continues for at least ten days after eclosion, while in D. melanogaster there is no increase in drosopterins after day 2-3 of adult life (Hadorn and Mitchell, 1951; Hadorn and Ziegler, 1958; Fuge, 1966; Fan et al, 1976).

In D. repleta, isoxanthopterin increased slightly from the beginning of pupation to the stage of development at which pupae have reddish-brown eyes. No additional increase in the amount of isoxanthopterin was observed in any stock until after eclosion. A small-moderate amount of isoxanthopterin was present in ar 12, while ar 4,

ar 5, and ar 6 had only trace-small amounts. Ar 11 had a small amount of isoxanthopterin, intermediate to amounts in ar 12 and the group ar 4, ar 5, and ar 6.

In contrast to the minor increases in isoxanthopterin during the pupal stage reported here and in a previous study of pteridines in D. repleta (Colley, 1967), D. melanogaster exhibits a rapid linear increase in isoxanthopterin content from the prepupal stage of development until the stage at which the pupal eyes begin ommochrome synthesis, after which there is a small general increase leading to a plateau shortly after eclosion (Hadorn and Mitchell, 1951; Fan et al, 1976). In addition, a distinct sexual dimorphism in the amount of isoxanthopterin appears earlier in D. melanogaster than in D. repleta. Isoxanthopterin levels are higher in males than in females as early as the late third instar larval stage in D. melanogaster. The difference becomes strikingly apparent at the prepupal stage and continues to be marked in adults (Hadorn and Mitchell, 1951). Colley (1967) reported that male and female third instar larvae of the original Atlanta stock of D. repleta (ar 1) exhibited no sexual dimorphism in any compound isolated. In the present study, examination of the expression of sexual dimorphism was restricted to the period after eclosion, and quantitative visual estimates from chromatograms of males and females of each stock at eclosion did not indicate sexual differences in any compound isolated (Tables 15-20). The absence of visually detectable sexual differences in pteridines during development and at eclosion in D. repleta constitutes a major species difference between D. repleta and D. melanogaster.

However, fluorimetric determination of isoxanthopterin concentration during aging (Tables 31-32; Figs. 4-6) revealed slight but significant quantitative sexual differences in isoxanthopterin concentration in ar 11 and ar 12 on the day of eclosion (E1).

During the pupal stage in ar 11 and ar 12, riboflavin disappeared and AHP-Biopterin increased to a small-moderate amount, while ar 4, ar 5, and ar 6 maintained trace amounts of riboflavin and small amounts of AHP-Biopterin. In addition, ar 11 and ar 12 had no P-6-COOH, sepiapterin, or isosepiapterin and trace to small amounts of P-6-CH_3 and kynurenine, while ar 4, ar 5, and ar 6 had trace amounts of P-6-COOH, sepiapterin, and isosepiapterin, and small-moderate amounts of P-6-CH_3 and kynurenine. UV-absorbing compounds were qualitatively and quantitatively similar in all stocks.

These results are in general agreement with those of Colley (1967) for the original Atlanta stock of D. repleta, but are quantitatively dissimilar to those reported for D. melanogaster (Hadorn and Mitchell, 1951; Hadorn and Ziegler, 1958; Fan et al, 1976). D. repleta, unlike D. melanogaster, does not exhibit a rapid accumulation of AHP-Biopterin during the pupal stage, or of sepiapterin prior to the onset of drosopterin synthesis. In D. repleta there is a relatively slow increase in AHP-Biopterin just prior to drosopterin synthesis, with little other fluctuation in amounts present until just prior to eclosion. The presence of sepiapterin and isosepiapterin in pupae of ar 4, ar 5, and ar 6 just prior to eclosion and their absence in ar 11 and ar 12, as well as the general rise in amounts of AHP-Biopterin in ar 11 and ar 12,

constitute additional differences among these stocks.

Fluorescent and UV-Absorbing Compounds in Adult Heads

Qualitative and quantitative differences in fluorescent and UV-absorbing compounds in heads of males and females of each stock during aging were slight, as were differences among stocks (Tables 21-25). The fluorescent compounds isolated in this study of male and female heads were qualitatively and quantitatively similar to those reported elsewhere for D. repleta (Throckmorton, 1962; Colley, 1967; Howell, 1969) and were qualitatively similar to those reported for D. melanogaster (Hadorn and Ziegler, 1958; Throckmorton, 1962; Wright and Hanly, 1966).

No difference between males and females in the amounts of drosopterin II or isosepiapterin was observed on the day of eclosion, as previously reported by Colley (1967). The only compound for which a qualitative difference between males and females was observed during aging was the unknown purple-fluorescing compound P III. On day E5, all stocks except ar 11 had trace levels of P III in females and none in males. Ar 11 exhibited no sexual differences in P III during aging.

Wright and Hanly (1966) reported slightly higher levels of drosopterins in heads of females than in males of wild type D. melanogaster of the same genotype, although the difference was not significant. Hadorn and Ziegler (1958) reported the level of isoxanthopterin in eyes of D. melanogaster males to be twice that in females. No such sexual differences in these compounds were found in any stock of

D. repleta in the present study, which is in agreement with results reported by Colley (1967). The continued synthesis of drosopterins until approxiamtely 10 days after eclosion in D. repleta contrasts with the cessation of drosopterin synthesis in D. melanogaster two to three days after eclosion (Hadorn and Ziegler, 1958; Fuge, 1966; Fan et al, 1976).

Qualitative and quantitative differences in the compounds present in heads of the five stocks of D. repleta are so slight and mixed in expression (Tables 21-25) that no grouping of stocks can be made based on pteridine patterns of adult heads. Thus, it would appear that phenotypic differences in adult eye color of ar 11, ar 12, and the group consisting of ar 4, ar 5, and ar 6 are attributable to biochemical differences other than qualitative or quantitative differences in pteridines of adult heads.

Fluorescent and UV-Absorbing Compounds in Adult Bodies

There are marked qualitative and quantitative differences in fluorescent and UV-absorbing compounds between male and female bodies of each stock during the aging period sampled, excluding the results for the actual day of eclosion (Tables 26-30). On the day of eclosion (E1), patterns of fluorescent and UV-absorbing compounds in bodies of males and females of a given stock of D. repleta are virtually indistinguishable both qualitatively and quantitatively. Similar findings for the original Atlanta stock of D. repleta were reported by Colley (1967). The failure to detect major sexual differences in the number and amounts of pteridines in the bodies of any D. repleta stock at eclosion is in

direct contrast to the sexual dimorphism found in studies of adults of D. melanogaster (Hadorn and Mitchell, 1951; Hadorn and Ziegler, 1958).

While pteridine patterns of male and female bodies within stocks are qualitatively and quantitatively virtually indistinguishable on day E1, differences between groups of stocks are apparent throughout the aging period. On day E1, the amount of isoxanthopterin in ar 12 exceeds that in ar 11, which is greater than the amount in ar 4, ar 5, and ar 6. Stock ar 4 is qualitatively distinguished from all other stocks on day E1 by the presence of the unknown green fluorescent compound G I.

Buzzati-Traverso (1953), in a paper chromatographic study of four phenotypically similar wild type stocks of D. melanogaster collected from different geographical locations, observed that bodies of flies of the same sex had similar but distinguishable patterns of fluorescent compounds, differing either in total number or in amounts of compounds present. These observations were based on comparisons of adults 14 days after eclosion since he had found that aging altered the quantities of a number of fluorescent compounds in female bodies, although not in male bodies, until approximately 12 days after eclosion.

The results of this study are generally supportive of the rather broad conclusion of Buzzati-Traverso that bodies of the same sex of different phenotypically wild type stocks of a species have similar but not identical patterns of fluorescent compounds. Stocks ar 4, ar 5, and ar 6 are phenotypically similar throughout the period of aging and are, in fact, very similar in patterns and amounts of fluorescent and UV-absorbing compounds. Adults of stocks ar 11 and ar 12 are

phenotypically dissimilar from each other and from all other stocks. Yet except for the presence in stocks ar 4, ar 5, and ar 6 of the unknown green fluorescent compound GI, which is absent in ar 11 and ar 12, and the presence of isosepiapterin in males of ar 11 and ar 12, but not in ar 4, ar 5, and ar 6 males, fluorescent and UV-absorbing compounds present in each sex are qualitatively similar in all stocks, although there are a number of distinct quantitative differences.

In contrast to Buzzati-Traverso's (1953) observations in D. melanogaster, aging was observed to alter the qualitative and quantitative pattern of fluorescent compounds in D. repleta male bodies, as well as in female bodies (Tables 26-30). This observation is in agreement with that reported previously by Colley (1967).

By day E5, chromatograms of male and female bodies are readily distinguishable in all stocks. Amounts of isoxanthopterin, xanthopterin, sepiapterin, AHP-Biopterin, and P-6-COOH in E5 male bodies far exceed the amounts present in females. In males of all stocks there is a consistent and obvious increase in amounts of isoxanthopterin, xanthopterin, P-6-COOH, sepiapterin, and AHP-Biopterin during aging, while females of all stocks show either a decrease or little change in the quantities of the same compounds (Tables 26-30). Bodies of males and females of each stock had similar quantitative increases in riboflavin during aging.

Buzzati-Traverso (1953) reported that aging of D. melanogaster females led to a decrease in a blue-violet compound which would appear

to be isoxanthopterin and increases in a yellow compound which probably was either riboflavin or sepiapterin and an "unknown" blue-fluorescing compound (presumably AHP or biopterin). A corresponding decrease in isoxanthopterin and increase in riboflavin were observed in females of all stocks of D. repleta during aging.

The increase in amounts of isoxanthopterin, xanthopterin, P-6-COOH, sepiapterin, isosepiapterin (not found in ar 4, ar 5, or ar 6), and AHP-Biopterin in male bodies during the aging period is consistent with the higher accumulation of pteridines reported in males of D. melanogaster, although there are distinct differences between species in rates of synthesis and/or accumulation of these compounds. The quantities of the above compounds were generally higher in ar 12 (Australia) than in ar 11 (Costa Rica) throughout the aging period. The rates of accumulation of these compounds during aging were similar in ar 4, ar 5, and ar 6, and levels were lower than observed in ar 11. Thus, there are also interstock differences in rates of synthesis and/or accumulation of these compounds. By day E15, bodies of ar 11 and ar 12 males had accumulated isoxanthopterin, P-6-COOH, and sepiapterin in excess of the amounts present in heads (cf. Tables 21-25 and Tables 26-30). Ar 4, ar 5, and ar 6 males accumulated either greater or approximately the same amounts of all the above compounds except isoxanthopterin in bodies as were found in heads, as has been reported for D. melanogaster (Hadorn and Mitchell, 1951; Ziegler and Harmsen, 1969).

While the above compounds are present at higher levels in ar 11 and ar 12, the unknown green-fluorescing compound G I, which increases

slightly in ar 4, ar 5, and ar 6 during the aging period, is absent in ar 11 and ar 12. G I appeared in ar 4 bodies on the day of eclosion. By day E5, G I was present in bodies of ar 5 and ar 6 as well as ar 4. In contrast to the generally higher levels of fluorescent compounds in male bodies than in female bodies of D. repleta, G I was present in higher concentrations in females than in males. In contrast to the slight rise in AHP and biopterin during aging in female bodies of D. melanogaster (Buzzati-Traverso, 1953), AHP-Biopterin disappeared during aging in females of D. repleta stocks ar 4, ar 5, and ar 6, and this decrease was temporally linked to the increase in G I. Females of ar 11 and ar 12 also exhibited a decrease in the amount of AHP-Biopterin during aging, although they lacked G I. The unknown green-fluorescing compound G I may be 2,4-dihydroxypteridine or a similar lumazine derivative. A xanthine oxidase-like enzyme has been isolated in D. melanogaster which catalyzes the conversion of 4-hydroxypteridine to 2,4-dihydroxypteridine (Forrest et al, 1961). 2,4-dihydroxypteridine has a green fluorescence and other characteristics similar to those of G I.

The synthesis of isoxanthopterin from 2-amino-4-hydroxypteridine (AHP) is dependent upon the presence of the enzyme xanthine oxidase (Krebs and Norris, 1949). Testicular tissue of larvae and adults of D. melanogaster is devoid of any xanthine oxidase activity, while the larval fat body and adult hemolymph have high activity (Ursprung and Hadorn, 1961; Munz, 1964). The qualitative and quantitative sexual differences in pteridine patterns of larvae, pupae, and adult bodies of D. melanogaster have been attributed to pteridine accumulation in testes

and their near absence in ovarian tissue (Hadorn and Mitchell, 1951; Hadorn and Ziegler, 1958; Ziegler and Harmsen, 1969). In studies of D. melanogaster male larval eye-imaginal buds and testes transplanted into female larvae, the levels of accumulation of isoxanthopterin by the transplants were found to be host-dependent, indicating that increased isoxanthopterin synthesis in males is not simply an intrinsic property of testicular tissue (Hadorn and Ziegler, 1958; Hadorn et al, 1958).

Throckmorton (1962) reported that testes of D. melanogaster at 10 days after eclosion had moderate amounts of isoxanthopterin, while D. repleta testes had small amounts. Yet he reported that the bodies of D. repleta had large amounts of isoxanthopterin, while D. melanogaster had only moderate amounts. Results of Throckmorton's study indicated that isoxanthopterin is present in other male body tissues and/or fluids of D. repleta besides testes. These results can be interpreted to indicate that isoxanthopterin is synthesized outside the testes and transported to these organs for storage or utilization, as has been reported for D. melanogaster. Due to the obvious species differences in timing of pteridine accumulation and the lack of xanthine oxidase electrophoretic and activity profiles for D. repleta tissues, it is uncertain at this time whether testicular tissue of D. repleta participates directly in the synthesis of isoxanthopterin or other pteridines found in male bodies.

GTP cyclohydrolase, the enzyme acting at the initial step in synthesis of pteridines from GTP, was recently isolated from a wild type

stock of D. melanogaster (Fan and Brown, 1976). These investigators reported that 80% of the enzyme activity was present in the heads of either sex. No sexual differences in enzyme activity were found in adults at one day after eclosion. Fan and Brown (1976) did not attempt to localize the enzyme activity found in bodies. Since they did not study isolated testicular tissues, their results provide no additional evidence as to whether the pteridines isolated from Drosophila testes in previous studies are produced in that tissue or are transported to testes from other sites of synthesis.

Fluorimetric Quantitation of Isoxanthopterin

A limited number of studies have utilized fluorimetric procedures to quantitate differences in amounts of pteridines present during development and/or aging of D. melanogaster (Hadorn and Mitchell, 1951; Wright and Hanly, 1966). Howell (1969) fluorimetrically quantitated pteridines chromatographically isolated from heads and decapitated bodies of 10-day-old males and females of an Atlanta wild type stock of D. repleta and silver nitrate-induced phenocopies.

In the present study, the effect of aging on isoxanthopterin content in male and female bodies was examined in the five wild type stocks of D. repleta. Isoxanthopterin was selected for fluorimetric quantitation both because visual estimates indicated striking differences in quantity among stocks and between sexes and marked changes in concentration during aging and because of its ready availability as a commercial standard and its stability during chemical manipulation.

Previous quantitative fluorimetric studies, instead of using individual pteridine standards, have made use of anthranilic acid as a reference standard for all pteridine determinations, so that comparisons with the results of this study are somewhat complicated. Hadorn and Mitchell (1951) reported an obvious sexual dimorphism in the amount of isoxanthopterin in a wild type stock of D. melanogaster during the prepupal and pupal stages of development. The amount of isoxanthopterin in male pupae, as well as in female pupae, increased at a near-linear rate from the late third instar larval stage until the segment of the pupal stage when the eyes are yellowish-brown in color. Subsequently, the amount of isoxanthopterin leveled off. While both sexes exhibited a linear increase in amount of isoxanthopterin during pupation, the rate of accumulation was much higher in males than in females. Fan et al (1976) also studied rates of synthesis of isoxanthopterin during D. melanogaster development, without regard to sex, and found a similar linear increase during pupal development. Hadorn and Mitchell (1951) reported that males of D. melanogaster 2-3 days after eclosion have a four-fold greater concentration of isoxanthopterin than do females of the same age. A linear rate of synthesis of AHP, sepiapterin, and drosopterins was also reported (Hadorn and Mitchell, 1951; Fan et al, 1976).

Howell (1969) quantitated a number of pteridines found in heads and decapitated bodies of 10-day-old males and females of a single Atlanta wild type stock of D. repleta. He noted that the most obvious sexual difference in quantity of pigment is in accumulation of

isoxanthopterin. He reported no noticeable qualitative differences and relatively few quantitative differences in pteridines of males and females. He reported a mean isoxanthopterin concentration in males of 3.5×10^{-3} µg/ml, as compared to 1.5×10^{-3} µg/ml in females, a concentration in males approximately three times that in females.

In the present study, statistically significant ($p < 0.001$) sexual differences in concentrations of isoxanthopterin were observed in all stocks by the fifth day after eclosion (Table 31). Stocks ar 11 and ar 12 differed from ar 4, ar 5, and ar 6 in that on the day of eclosion (E1) there was already a statistically significant sexual difference in isoxanthopterin concentration (Table 31; Figs. 4 and 5). Isoxanthopterin concentration increased linearly in males of all stocks until at least 10 days after eclosion (Fig. 4). At 15 days after eclosion (E15), males of ar 5, ar 11, and ar 12 appeared to be approaching a plateau in the accumulation of isoxanthopterin (Fig. 4).

Comparison of the 95% confidence intervals of the mean isoxanthopterin concentrations for males of each stock on each day sampled during the aging period reveals overlap for stocks ar 4, ar 5, and ar 6. The 95% confidence intervals of the mean for ar 11 overlap no other stock during the aging period, except for stock ar 5 on day E10. The 95% confidence intervals of the mean for ar 12 do not overlap any other stock at any sampling day during the aging period (Fig. 4).

In females of all stocks there was a decrease in isoxanthopterin concentration until approximately 10 days after eclosion (E10), at which time the concentration had stabilized at a minimum level (Fig. 5;

Table 31). Females of ar 11 and ar 12 on day E1 have higher concentrations of isoxanthopterin than do females of any other stock. Females of ar 11 are significantly different from all other stocks on day E5 (the 95% confidence interval of the mean isoxanthopterin concentration does not overlap that of any other stock). By day E10, females of all stocks have an overlap of 95% confidence intervals of the means.

Results of this study indicate a ratio of male-to-female isoxanthopterin concentration as great as 467 to 1, in contrast to the ratios in previous quantitative fluorimetric studies in D. melanogaster and D. repleta, in which the isoxanthopterin concentration in males was approximately three times that in females. The greater order of magnitude of sexual difference in isoxanthopterin concentration in this study is probably in part attributable to the use of isoxanthopterin itself instead of anthranilic acid as a standard, suggesting that quantitative sexual differences in isoxanthopterin (and possibly in other pteridines) are considerably greater than previously indicated.

Quantitative visual estimates of pteridines in bodies of the five stocks (Tables 26-30) indicated an approximately linear rise in AHP-Biopterin, xanthopterin, sepiapterin, P-6-COOH, and riboflavin in males during the aging period in which isoxanthopterin concentration was measured fluorimetrically. Thus, the increase in pteridines during aging of adults reported here is comparable to the increase in pteridines reported during the pupal stage in D. melanogaster (Hadorn and Mitchell, 1951; Fan et al, 1976). The decrease in isoxanthopterin concentration

in females during the aging period (Table 31; Fig. 5), with a stabilization at approximately day E10, is in agreement with Buzzati-Traverso's (1953) observation based on visual estimates that isoxanthopterin concentration in D. melanogaster females decreases during aging until approximately 12 days after eclosion, at which time it stabilizes at a trace level.

The observation that isoxanthopterin concentration increases during aging in males of all stocks and simultaneously decreases in females of all stocks (Figs. 4 and 5; Table 31) indicates that sexual differences in the accumulation of isoxanthopterin depend not only on the presence of a storage site for isoxanthopterin in males, but also on a distinct sexual difference in rates of synthesis. The visually quantitated differences in a number of the other pteridines (Tables 26-30) also indicate distinct sexual differences in rates of production and accumulation in males and females of all stocks.

Pteridine Metabolism

Throckmorton (1962) proposed that the organization of the metabolic system governing pteridine metabolism in Drosophila encompasses at least three subdivisions: (1) a basic biosynthetic pathway, which could be similar in all species of Drosophila; (2) separate control mechanisms governing synthesis of pteridines in different organs or tissues; and (3) control mechanisms for accumulation of pteridines in different organs (controlling rates of turnover, availability of sites of pigment deposition, etc.). The last two groups of mechanisms

or levels of control of pteridine metabolism could be expected to vary between species and between sexes within each species. Results of this study are consistent with the control of pteridine metabolism at each of these levels.

All five stocks of D. repleta have a basic pteridine pattern which qualitatively resembles the pattern described for D. melanogaster. There are obvious differences between the two species as to timing of appearance and accumulation of pteridines and rates of synthesis during development and aging, as well as differences among stocks of D. repleta, but the basic pteridine patterns are not greatly dissimilar.

Previous studies with mutants of D. melanogaster which affect pteridine synthesis have demonstrated that certain pteridines fail to appear in mutants, others are present at reduced levels, and others are present in increased amounts (Hadorn and Mitchell, 1951; Forrest et al, 1956; Hadorn and Ziegler, 1958). Results of studies of mutant and wild type D. melanogaster have been the basis for proposal of a number of reaction sequences in pteridine metabolism (Forrest and Mitchell, 1954c; Taira, 1960; Hubby and Throckmorton, 1960; Throckmorton, 1962; Ziegler and Harmsen, 1969).

A basic metabolic system governing pteridine metabolism should proceed in a stepwise manner with an orderly sequence of transformations. Each step in the pathway should be related to a specific precursor or groups of similar precursors which are acted on by specific enzymes, converting the precursors to a specific product or group of products.

Only two enzymes have been studied in D. melanogaster which are known to participate in the basic metabolic pathway of pteridine synthesis, xanthine oxidase and GTP cyclohydrolase. In a recent set of papers, the presence, purification, properties, and correlation of enzyme activity with synthesis of pteridines during development of wild type D. melanogaster were reported for the enzyme GTP cyclohydrolase (Burg and Brown, 1968; Fan and Brown, 1976; Fan et al, 1976). GTP cyclohydrolase catalyzes the conversion of GTP to the initial pteridine intermediate dihydroneopterin triphosphate (Burg and Brown, 1968; Wolf and Brown, 1969; Brown, 1970; Shiota et al, 1970). The fluctuations in ATP-GTP and guanine-xanthine during development in all stocks of D. repleta (Tables 18-20) may reflect a precursor role of GTP in pteridine synthesis in this species.

The appearance of AHP-Biopterin prior to drosopterin synthesis during the pupal stage in all stocks of D. repleta supports the conversion sequence in the pathway of drosopterin synthesis (biopterin → sepiapterin → drosopterin) proposed by Ziegler and Hadorn (1958), Taira (1960), and Hubby and Throckmorton (1960). The absence of sepiapterin in stocks ar 11 and ar 12 prior to eclosion and the higher levels of AHP-Biopterin and slightly lower levels of drosopterins in these stocks than in ar 4, ar 5, and ar 6 might be interpreted to imply a slower rate of conversion of biopterin via the intermediate sepiapterin to drosopterins in these two stocks. Alternatively, the failure to detect sepiapterin in ar 11 and ar 12 prior to eclosion may indicate that its

conversion to drosopterins is so rapid that no detectable quantity of the compound accumulates at this stage. The fact that sepiapterin was present in heads of all stocks on the day of eclosion (Tables 26-30) tends to support this premise, rather than a block or reduced rate of biopterin conversion. The general increase in AHP-Biopterin, sepiapterin, isosepiapterin, and xanthopterin in adult heads during aging (Tables 21-25) may reflect accumulation of drosopterin and/or isoxanthopterin precursors which are no longer being converted to end products. Thus, an enhanced rate of synthesis of precursors and intermediates exceeding the rate of their conversion may explain the rise of one or more of these compounds.

Recent studies of the xanthine oxidase enzyme system (Rembold, 1970; Harmsen, 1970) indicate that in different organs or tissues the enzyme system may be regulated by separate or compartmentalized control mechanisms, as suggested by Throckmorton (1962). Xanthine oxidase functions in conversion of a number of the simple pterines (e.g., AHP to isoxanthopterin, 7,8-dihydropteridine to 7,8-dihydroxyxanthopterin, xanthopterin to leucopterin) and is capable of catalyzing the oxidation of the C-2, C-4, C-6, and C-7 positions of the pteridine nucleus. The enzyme will catalyze the oxidation of the C-6 position only if the precursor is 7,8-dihydropteridine (Rembold, 1970). Thus, the extent of oxidation of the C-6 position and the specific nature of the product depend on the state of reduction of the substrate.

The visually quantitated levels of pteridines in heads and bodies of all five stocks of D. repleta indicate that different metabolic

control mechanisms are active in heads and bodies. In addition to the obvious restriction of drosopterins to heads, xanthopterin and isoxanthopterin, two compounds which are end products of simple pteridine metabolism, show marked differences in accumulation in heads and bodies. Heads of all stocks have amounts of xanthopterin far in excess of isoxanthopterin, while in bodies the reverse is true. The increase in xanthopterin in heads, with no change in amount of isoxanthopterin (Tables 21-25), and the increase in isoxanthopterin in male bodies, with a much slower increase in xanthopterin (Tables 26-30), support a difference in the chemical nature of the tissue environment of heads and bodies.

In support of Throckmorton's (1962) third proposed level of control of pteridine metabolism, that of accumulation in different organs, are the obvious sexual difference in isoxanthopterin concentration and the differences between males and females of all stocks in the amounts of a number of other pteridines. Obviously, a sexual difference also exists in rates of synthesis and accumulation of these compounds in one or more tissues or organs. Whether these sexual differences are tissue-specific, organ-specific, or organism-specific remains to be determined.

CONCLUSIONS

Atlanta, Hawaii, and Yucatan stocks of D. repleta differ little either qualitatively or quantitatively in pteridines and UV-absorbing compounds, and differences in isoxanthopterin concentration are not significant. Isoxanthopterin concentration in adult male bodies is significantly greater in Costa Rica than in Atlanta, Hawaii, and Yucatan, and is significantly greater in Australia than in any other stock. Visual estimates indicate similarly elevated levels of other pteridines in Costa Rican and Australian male bodies. Sexual differences in pteridines are not visually detectable at eclosion but are marked in all stocks five days later. Isoxanthopterin increases significantly during aging in males and decreases significantly in females. Visual estimates indicate similar changes in other pteridines during aging. Atlanta, Hawaii, and Yucatan are similar in morphology and developmental timing and interbreed freely with each other and a recently isolated Atlanta stock. Costa Rica and Australia complete the life cycle 24-48 hours earlier than other stocks, are reproductively isolated from each other and all other stocks, and differ in eye and body pigmentation from other stocks and the classic species description. Differences in final phenotypic expression of adult eye color do not appear to be due to differences in pteridine patterns of adult heads. Costa Rica (scarlet eyes) and Australia (brick-red eyes) exhibit no distinct qualitative or quantitative differences in pteridines from the

Atlanta-Hawaii-Yucatan group (sepia eyes). Results do suggest that variation in eye color may reflect differences in the metabolic fate of kynurenine in the pathway of ommochrome synthesis. The brighter yellow coloration of testes in Australia is tentatively attributed to a higher concentration of sepiapterin than in other stocks.

While statements concerning the pathway from pteridine precursors to synthesis of drosopterins in D. repleta must be tentative, the results support certain conclusions relating to proposed pathways of pteridine synthesis in Drosophila. Pteridine synthesis during embryonic and larval life appears to be restricted primarily to reaction sequences leading to simple pterine catabolites, i.e., biopterin, isoxanthopterin, and xanthopterin. Relative times of appearance of these pteridines are consistent with the proposed reaction sequence biopterin → AHP → isoxanthopterin and/or xanthopterin. At the time in the pupal stage when ommochromes are deposited as eye pigments, there appears to be a "switch" in pteridine metabolism in the direction of synthesis of polyhydroxy-substituted pteridines, converting biopterin via sepiapterin to the drosopterins, with a temporary plateau in rates of conversion of biopterin to isoxanthopterin and xanthopterin via AHP. The simultaneous fluctuations in (a) types and amounts of pteridines synthesized, (b) amounts of purines present, and (c) riboflavin content suggest that an allosteric control mechanism, influenced by riboflavin availability, operates to shift the activity of xanthine oxidase from simple pterine synthesis early in development to synthesis of substituted pteridines during the pupal stage.

CHAPTER VI

RECOMMENDATIONS

A number of areas of investigation are recommended which could provide answers to questions raised by results of this study.

1. Transplantation of male larval Malpighian tubules and testes into female larvae and subsequent qualitative evaluation and fluorimetric quantitation of pteridines in transplant individuals and their tissues during development.
2. Comparative evaluation of xanthine oxidase profile, including electrophoretic analysis of isozymes and assay of enzyme activity, for whole organisms and specific organs during development and aging.
3. Analysis of GTP cyclohydrolase activity profile during development and aging and organ-specific analysis in one or more of the D. repleta stocks.
4. Fluorimetric quantitation of individual pteridines other than isoxanthopterin during development and aging in one or more of the D. repleta stocks.
5. A more extensive evaluation of reproductive success between recently isolated wild type stocks, including Australian and Costa Rican stocks.
6. Quantitative evaluation of pteridine patterns of interstock hybrids resulting from successful crosses between stocks.

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APPENDIX A

DESIGN AND UTILIZATION OF OVIPOSITION CHAMBERS

Oviposition Unit Type I

The end was removed from a 4 oz plastic vial (Carolina Biological Supply Company). One end was closed with a polypropylene foam plug to allow air exchange, while the other end was secured with aluminum wire screening soldered to the plastic. One to three such units were used per collection, depending on the average number of eggs laid by the stock in question. Vials were stocked with approximately 20 males and 40 females. Two sizes of disposable plastic petri dishes were used as containers of food serving as oviposition surfaces. Falcon No. 1034 Rodak plates (65 x 15 mm) were used to accept a single oviposition vial. Sterile, disposable 100 x 15 mm dishes (Fisher Scientific Company) were used when more than one oviposition vial was needed. All oviposition vials and food dishes were covered with one-quart capacity plastic containers to prevent contamination of the oviposition surface by eggs of flies which had escaped into the laboratory. Vials were set on fresh medium for 24 hr intervals and were removed and cleared of adhering medium and eggs before being transferred to fresh oviposition surfaces. Petri dishes from which vials had been removed after 24 hr oviposition intervals were covered, and eggs were allowed to develop.

Oviposition Unit Type II

To provide sufficient numbers of organisms for sampling in sequential developmental analysis, it was necessary to collect large

numbers of eggs during each 24 hr interval. The rate of oviposition in the most fecund stocks of D. repleta in this study (Australia and Costa Rica) is somewhat lower than in D. melanogaster and is very much lower in the Atlanta stock. The small populations in Oviposition Unit Type I yielded insufficient numbers of eggs; thus, a second type of oviposition unit was constructed. One-quart clear plastic containers were fitted with aluminum wire mesh bases. An opening was made in the opposite end to allow air transfer and a polypropylene foam plug was inserted in the opening to prevent escape of flies. These chambers fitted snugly into 150 x 15 mm petri dishes used to contain oviposition medium and were covered to prevent possible contamination. Type II chambers housed populations of approximately 100 males and 200 females. While these chambers were quite satisfactory for use as long-term oviposition chambers (Humphrey, 1974) and made possible the collection of larger numbers of eggs during each 24 hr period, they still had the disadvantage that medium and eggs adhered to the screen surface, so that considerable time was required for cleaning before the chambers could be transferred to fresh food surfaces.

Oviposition Unit Type III

Disadvantages of previously described oviposition chambers which were considerations in the design of the final oviposition units used in this study are listed below.

(1) Time required for clearing adhering medium and eggs from the aluminum screening.

(2) A rate of death of parental adults as great as ten per cent per day due to entrapment in the medium.

(3) Wetting of adult wings, resulting in adherence to bodies in such a manner that eggs could not be deposited or were reduced in number.

(4) Total time required for handling of five stocks in preparation for the next day's food dishes.

(5) The requirement of an additional cover over the units to insure that all eggs on the oviposition surface were those of the stock under study, and not those of other flies present in the laboratory.

(6) The distribution of eggs over a large surface area, requiring considerable time to (a) determine approximate numbers of eggs present and (b) collect eggs.

An opening 60 mm in diameter was cut with a circular saw in the bottom of a 18.5 x 13.5 x 5.0 cm plastic container (Vlchek Plastic Company). A Falcon No. 1034 Rodak plate containing food could be placed to fit snugly in this opening.

These units had a number of advantages over the other designs. The petri dish for each unit could be changed daily without contact with screening material or with the surface of the oviposition unit itself, thus eliminating the time-consuming process of cleaning eggs and medium from the screen covers. Daily transfers of these units for all stocks required a maximum of 25 min, as compared to times in excess of an hour for the other types. Type III units could be stocked with larger numbers of flies without crowding and provided a dry surface on which adults could rest, groom, and copulate without coming into

contact with a wetted surface. Each unit was stocked with approximately 200 males and 400 females. Concentrating the surface area of the food containers allowed for ready viewing and more rapid collection of eggs laid by the larger population. No additional external covering of the units was necessary since the unit itself was a closed chamber, with no sizeable opening through which females outside the chamber could introduce eggs of stocks not under study.

APPENDIX B

Table 1. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 4: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AHP-BIO	0	0	0	+	±	±	+	+	+	+	±	+	+	2+	2+	3+
DRO I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	3+
DRO II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
DRO III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	4+
ISOSP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
ISOX	±	±	±	+	±	±	±	±	±	+	±	+	+	2+	2+	2+
KA	±	±	±	+	+	+	±	±	±	±	+	+	+	+	+	+
KYN	±	±	±	2+	3+	3+	3+	4+	5+	5+	3+	3+	3+	4+	4+	4+
P-6-CH_3	0	0	0	+	3+	3+	3+	3+	2+	+	2+	3+	3+	4+	4+	4+
P-6-COOH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
RIB	±	±	±	3+	4+	4+	5+	5+	4+	4+	3+	3+	3+	2+	2+	+
SP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
XAN	±	±	±	+	+	+	+	+	+	+	+	+	+	+	+	+
XIC	0	0	0	0	±	±	+	+	+	+	+	+	+	+	+	+

Table 1. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 4: Daily Sequential Analysis, Continued

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BY I	0	0	0	3+	+	+	+	+	+	±	+	+	+	+	+	±
BY II	0	0	0	2+	+	±	±	±	0	0	0	0	0	0	0	0
G II	±	±	±	3+	+	+	±	0	0	0	0	0	0	0	0	0
P I	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+
P II	0	0	0	±	+	2+	+	+	+	±	0	0	0	0	0	0
P III	±	±	±	+	2+	3+	2+	+	+	+	2+	2+	2+	3+	2+	2+
WB	0	0	0	2+	2+	2+	2+	+	±	±	±	±	±	+	+	+
W I	0	0	0	+	+	+	+	±	0	0	0	0	0	0	0	0
W II	0	0	0	+	+	±	±	±	±	0	0	0	0	0	0	0
W III	0	0	0	+	+	+	+	±	0	0	0	0	0	0	0	0
Y I	±	±	±	0	0	0	+	+	+	+	0	0	0	0	0	+
Y II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
Y III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
Y IV	0	0	0	+	+	+	+	+	±	0	0	0	0	0	0	0

Table 2. Visually Estimated Quantities of UV-Absorbing Compounds Isolated from Developmental Stages of ar 4: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AD-A	0	0	0	0	±	±	±	±	0	0	0	0	0	±	0	±
5'-AMP	+	+	+	3+	3+	3+	2+	+	±	±	±	+	+	+	+	+
ATP-GTP	3+	3+	3+	4+	4+	4+	4+	3+	2+	+	+	+	+	+	±	±
GUAN-XA	0	0	0	2+	3+	3+	3+	2+	±	±	±	±	+	2+	2+	±
HXA	0	0	0	0	+	+	+	+	±	0	+	2+	+	0	±	±
IMP	+	+	+	3+	3+	2+	2+	+	±	±	0	0	0	0	0	+
UA	±	±	±	3+	4+	4+	4+	3+	2+	+	+	+	2+	2+	2+	3+
UV I	0	0	0	4+	3+	+	±	0	0	0	0	0	0	0	0	0
UV II	0	0	0	±	±	±	0	0	0	0	0	0	0	0	0	0

Table 3. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 5: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AHP-BIO	0	0	+	+	±	±	+	+	+	+	±	+	+	2+	2+	3+
DRO I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	3+
DRO II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
DRO III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	4+
ISOSP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
ISOX	±	±	+	+	±	±	±	±	±	+	±	+	+	2+	2+	2+
KA	±	±	+	+	+	+	±	±	±	±	+	+	+	+	+	+
KYN	±	±	+	2+	3+	3+	3+	4+	5+	5+	3+	3+	3+	4+	4+	4+
P-6-CH_3	0	0	±	+	3+	3+	3+	3+	2+	+	2+	3+	3+	4+	4+	4+
P-6-COOH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
RIB	±	+	2+	3+	4+	4+	5+	5+	4+	4+	3+	3+	3+	3+	2+	+
SP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
XAN	±	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+
XIC	0	0	0	0	±	±	+	+	+	+	+	+	+	+	+	+

Table 3. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 5: Daily Sequential Analysis, Continued

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BY I	0	0	2+	3+	+	+	+	+	+	±	+	+	+	+	+	±
BY II	0	0	2+	2+	+	±	±	±	0	0	0	0	0	0	0	0
G II	±	±	2+	3+	+	+	±	0	0	0	0	0	0	0	0	0
P I	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P II	0	0	±	±	+	2+	+	+	+	±	0	0	0	0	0	0
P III	±	±	+	+	2+	3+	2+	+	+	+	2+	2+	2+	3+	2+	2+
WB	0	0	+	2+	2+	2+	2+	+	±	±	±	±	±	+	+	+
W I	0	0	±	+	+	+	+	±	0	0	0	0	0	0	0	0
W II	0	0	+	+	±	±	±	±	±	0	0	0	0	0	0	0
W III	0	0	+	+	+	+	+	±	0	0	0	0	0	0	0	0
Y I	±	±	0	0	0	0	+	+	+	+	0	0	0	0	0	+
Y II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
Y III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
Y IV	0	0	+	+	+	+	+	+	±	0	0	0	0	0	0	0

Table 4. Visually Estimated Quantities of UV-Absorbing Compounds Isolated from Developmental Stages of ar 5: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AD-A	0	0	0	0	±	±	±	±	0	0	0	0	0	±	0	±
5'-AMP	+	+	3+	3+	3+	3+	2+	+	±	±	±	+	+	+	+	+
ATP-GTP	3+	3+	4+	4+	4+	4+	4+	3+	+	+	+	+	+	+	±	±
GUAN-XA	0	0	2+	2+	3+	3+	3+	2+	±	±	±	±	+	2+	2+	±
HXA	0	0	0	0	+	+	+	+	±	0	+	2+	+	0	±	±
IMP	+	+	+	3+	3+	2+	+	±	±	±	0	0	0	0	0	+
UA	±	±	2+	3+	4+	4+	4+	3+	2+	+	+	+	2+	2+	2+	3+
UV I	0	0	3+	4+	3+	+	±	0	0	0	0	0	0	0	0	0
UV II	0	0	±	±	±	±	0	0	0	0	0	0	0	0	0	0

Table 5. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 6: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AHP-BIO	0	0	+	+	±	±	+	+	+	+	±	+	+	2+	2+	3+
DRO I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	3+
DRO II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
DRO III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	4+
ISOSP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
ISOX	±	±	+	+	±	±	±	±	±	+	±	+	+	2+	2+	2+
KA	±	±	+	+	+	+	±	±	±	±	+	+	+	+	+	+
KYN	±	±	+	2+	3+	3+	3+	4+	5+	5+	3+	3+	3+	4+	4+	4+
P-6-CH_3	0	0	±	+	3+	3+	3+	3+	2+	+	2+	3+	3+	4+	4+	4+
P-6-COOH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
RIB	±	±	2+	3+	4+	4+	5+	5+	4+	4+	3+	3+	3+	3+	2+	+
SP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
XAN	±	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+
XIC	0	0	0	0	±	±	+	+	+	+	+	+	+	+	+	+

Table 5. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 6: Daily Sequential Analysis, Continued

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BY I	0	0	2+	3+	+	+	+	+	+	±	+	+	+	+	+	±
BY II	0	0	2+	2+	+	±	±	±	0	0	0	0	0	0	0	0
G II	±	±	2+	3+	+	+	±	0	0	0	0	0	0	0	0	0
P I	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P II	0	0	±	±	+	2+	+	+	+	±	0	0	0	0	0	0
P III	±	±	+	+	2+	3+	2+	+	+	+	2+	2+	2+	3+	2+	2+
WB	0	0	+	2+	2+	2+	2+	+	±	±	±	±	±	+	+	+
W I	0	0	±	+	+	+	+	±	0	0	0	0	0	0	0	0
W II	0	0	+	+	±	±	±	±	±	0	0	0	0	0	0	0
W III	0	0	+	+	+	+	+	±	0	0	0	0	0	0	0	0
Y I	±	±	0	0	0	0	+	+	+	+	0	0	0	0	0	+
Y II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
Y III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
Y IV	0	0	+	+	+	+	+	+	±	0	0	0	0	0	0	0

Table 6. Visually Estimated Quantities of UV-Absorbing Compounds Isolated from Developmental Stages of ar 6: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AD-A	0	0	0	0	±	±	±	±	0	0	0	0	0	±	0	±
5'-AMP	+	+	3+	3+	3+	3+	2+	+	±	±	±	+	+	+	+	+
ATP-GTP	3+	3+	4+	4+	4+	4+	4+	3+	+	+	+	+	+	+	±	±
GUAN-XA	0	0	2+	2+	3+	3+	3+	2+	±	±	±	±	+	2+	2+	±
HXA	0	0	0	0	+	+	+	+	±	0	+	2+	+	0	±	±
IMP	+	+	+	3+	3+	2+	+	±	±	±	0	0	0	0	0	+
UA	±	±	2+	3+	4+	4+	4+	3+	2+	+	+	+	2+	2+	2+	3+
UV I	0	0	3+	4+	3+	+	±	0	0	0	0	0	0	0	0	0
UV II	0	0	±	±	±	±	0	0	0	0	0	0	0	0	0	0

Table 7. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 11: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
AHP-BIO	0	±	+	+	±	+	+	+	+	±	+	+	2+	3+	4+
DRO I	0	0	0	0	0	0	0	0	0	0	0	0	0	+	3+
DRO II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
DRO III	0	0	0	0	0	0	0	0	0	0	0	0	0	+	4+
ISOSP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ISOX	±	+	2+	2+	+	+	+	+	2+	+	2+	2+	3+	3+	3+
KA	±	+	+	+	+	+	+	+	+	+	+	2+	3+	2+	+
KYN	±	+	2+	2+	3+	3+	4+	4+	5+	3+	3+	3+	3+	2+	+
P-6-CH_3	0	+	+	+	+	3+	3+	2+	+	2+	3+	3+	3+	+	+
P-6-COOH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIB	±	+	2+	2+	3+	4+	4+	3+	3+	2+	2+	2+	2+	±	0
SP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
XAN	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+
XIC	0	0	0	0	±	±	+	+	+	+	+	2+	3+	+	+

Table 7. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 11: Daily Sequential Analysis, Continued

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
BY I	0	3+	3+	2+	2+	+	+	+	±	±	+	+	+	+	±
BY II	0	3+	2+	2+	2+	±	±	0	0	0	0	0	0	0	0
G II	±	2+	3+	3+	+	±	0	0	0	0	0	0	0	0	0
P I	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P II	0	0	0	+	2+	2+	+	±	0	0	0	0	0	0	0
P III	±	+	+	2+	3+	3+	3+	+	+	2+	2+	2+	3+	3+	2+
WB	0	+	2+	3+	3+	2+	+	+	±	±	±	±	±	±	+
W I	0	+	+	+	+	+	±	0	0	0	0	0	0	0	0
W II	0	+	+	+	+	+	±	0	0	0	0	0	0	0	0
W III	0	+	+	+	+	+	+	±	0	0	0	0	0	0	0
Y I	±	0	0	0	0	+	+	+	+	0	0	0	0	0	+
Y II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y IV	0	+	2+	+	+	+	+	±	0	0	0	0	0	0	0

Table 8. Visually Estimated Quantities of UV-Absorbing Compounds Isolated from Developmental Stages of ar 11: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
AD-A	0	0	0	±	±	±	0	0	0	0	0	0	±	0	±
5'-AMP	+	2+	3+	3+	4+	3+	2+	+	±	+	+	±	+	+	+
ATP-GTP	3+	4+	4+	4+	4+	4+	3+	2+	+	+	+	±	±	±	±
GUAN-XA	0	+	2+	2+	3+	3+	2+	2+	±	2+	+	+	+	+	±
HXA	0	0	0	0	+	+	+	±	0	+	2+	+	0	±	±
IMP	+	2+	3+	3+	3+	3+	+	+	±	0	0	0	0	0	+
UA	0	+	3+	3+	4+	4+	3+	2+	2+	2+	3+	2+	3+	3+	3+
UV I	0	3+	4+	4+	3+	±	0	0	0	0	0	0	0	0	0
UV II	0	±	±	±	±	0	0	0	0	0	0	0	0	0	0

Table 9. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 12: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
AHP-BIO	0	±	+	2+	+	+	+	+	+	±	±	+	2+	3+	4+
DRO I	0	0	0	0	0	0	0	0	0	0	0	0	0	+	3+
DRO II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
DRO III	0	0	0	0	0	0	0	0	0	0	0	0	0	+	4+
ISOSP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ISOX	±	+	2+	3+	+	+	+	2+	2+	2+	3+	3+	4+	4+	4+
KA	±	+	+	+	±	±	±	±	±	±	+	+	2+	2+	+
KYN	±	+	2+	2+	3+	2+	4+	4+	2+	2+	2+	3+	4+	2+	+
P-6-CH_3	0	+	+	+	+	3+	3+	+	+	2+	2+	3+	3+	2+	+
P-6-COOH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIB	±	±	+	+	2+	3+	3+	2+	2+	2+	2+	2+	2+	+	0
SP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
XAN	±	±	+	2+	+	+	+	+	+	+	+	+	+	+	+
XIC	0	0	0	0	±	±	+	+	+	+	+	2+	2+	+	+

Table 9. Visually Estimated Quantities of Fluorescent Compounds Isolated from Developmental Stages of ar 12: Daily Sequential Analysis, Continued

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
BY I	0	3+	3+	2+	+	+	+	+	±	±	+	+	+	+	±
BY II	0	3+	2+	2+	+	±	0	0	0	0	0	0	0	0	0
G II	±	2+	3+	3+	+	±	0	0	0	0	0	0	0	0	0
P I	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P II	0	0	0	+	2+	+	+	±	0	0	0	0	0	0	0
P III	±	+	+	2+	3+	3+	3+	+	+	+	2+	2+	3+	3+	2+
WB	0	+	2+	3+	3+	2+	+	+	±	±	±	±	±	±	+
W I	0	+	+	+	+	+	±	0	0	0	0	0	0	0	0
W II	0	±	+	+	+	+	±	0	0	0	0	0	0	0	0
W III	0	±	+	+	+	+	+	±	0	0	0	0	0	0	0
Y I	±	0	0	0	0	+	+	+	+	0	0	0	0	0	+
Y II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y IV	0	+	2+	+	+	+	+	±	0	0	0	0	0	0	0

Table 10. Visually Estimated Quantities of UV-Absorbing Compounds Isolated from Developmental Stages of ar 12: Daily Sequential Analysis

Compound	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
AD-A	0	0	0	0	±	±	±	0	0	0	0	0	±	0	±
5'-AMP	+	2+	3+	3+	4+	3+	2+	+	±	+	+	±	+	+	+
ATP-GTP	3+	4+	4+	4+	4+	4+	3+	2+	+	+	+	±	±	±	±
GUAN-XA	0	+	2+	2+	3+	3+	2+	2+	±	2+	+	+	+	+	±
HXA	0	0	0	0	+	+	+	±	0	+	2+	+	0	±	±
IMP	+	2+	3+	3+	3+	3+	+	+	±	0	0	0	0	0	+
UA	0	+	2+	3+	4+	4+	3+	2+	2+	2+	3+	3+	2+	3+	3+
UV I	0	3+	4+	4+	3+	±	0	0	0	0	0	0	0	0	0
UV II	0	+	±	±	±	0	0	0	0	0	0	0	0	0	0

Table 11. Concentrations of Isoxanthopterin Isolated from Male Bodies, Calculated from Fluorimetric Readings of Individual Samples and Expressed as µg/mg Wet Weight of Sample

Day	Isoxanthopterin Concentration				
	ar 4	ar 5	ar 6	ar 11	ar 12
E1	0.0036	0.0046	void	0.0125	0.0636
	0.0030	0.0035	0.0033	0.0106	0.0614
	0.0025	0.0028	0.0025	0.0091	0.0880
	0.0029	void	0.0049	0.0106	void
	0.0030	0.0050	0.0036	0.0144	0.0604
	0.0033	0.0031	0.0045	0.0103	0.0891
	0.0032	0.0041	0.0047	0.0143	0.0514
	0.0030	void	0.0055	0.0135	0.0574
	0.0041	0.0015	0.0063	void	0.0652
	(N = 10)	(N = 8)	(N = 9)	(N = 9)	(N = 9)
E5	0.0696	0.1250	0.0899	0.1698	0.2811
	0.0980	0.0787	0.0379	0.1550	0.2294
	0.0752	0.0435	0.0809	0.1698	0.2778
	0.0685	0.1318	0.0598	0.1433	0.2453
	0.0645	0.0795	0.0282	0.1484	0.2734
	0.0875	0.0546	0.0642	0.1374	0.2014
	0.0440	0.0650	0.0144	0.1585	0.2412
	0.0542	0.1119	0.0614	0.1371	0.2877
	0.0475	0.0914	0.0719	0.1393	0.2263
	0.0547	0.0896	0.0462	0.1645	0.2925
	(N = 10)	(N = 10)	(N = 10)	(N = 10)	(N = 10)

Table 11. Concentrations of Isoxanthopterin Isolated from Male Bodies, Calculated from Fluorimetric Readings of Individual Samples and Expressed as µg/mg Wet Weight of Sample, Continued

Day	Isoxanthopterin Concentration				
	ar 4	ar 5	ar 6	ar 11	ar 12
E10	0.1471	0.3075	0.2228	0.2268	0.4258
	0.1583	0.1872	0.1215	0.3117	0.4204
	0.1540	0.2141	0.1512	0.2153	0.4870
	0.1326	0.1957	0.1794	0.2731	0.4545
	0.0729	0.2229	0.0850	0.2632	0.4131
	0.0981	0.2749	0.1152	0.2710	0.4555
	0.1547	0.1928	0.1395	0.2515	0.4487
	0.0571	0.2265	0.1726	0.2670	0.4255
	0.1744	0.1667	0.0575	0.2689	0.4656
	0.0780	0.1709	0.2763	0.2690	0.5065
	(N = 10)	(N = 10)	(N = 10)	(N = 10)	(N = 10)
E15	0.3085	0.3094	0.2571	0.3774	0.5727
	void	0.3553	0.1965	0.4224	0.5021
	void	0.1988	0.1909	0.2907	0.5335
	0.2097	0.2315	0.2428	0.3561	0.5147
	0.2159	0.2653	0.2750	0.3075	0.4498
	0.2188	0.2113	0.2459	0.3141	0.4201
	0.2346	0.2808	0.2011	0.4484	0.5380
	0.2352	0.2388	0.1976	0.3160	0.4149
	0.2353	0.2751	0.2048	0.3216	0.4724
	0.1952	0.2806	0.2028	0.3175	0.5403
	(N = 8)	(N = 10)	(N = 10)	(N = 10)	(N = 10)

Table 12. Concentrations of Isoxanthopterin Isolated from Female Bodies, Calculated from Fluorimetric Readings of Individual Samples and Expressed as µg/mg Wet Weight of Sample

Day	Isoxanthopterin Concentration ar 4	ar 5	ar 6	ar 11	ar 12
E1	0.003419	0.003333	0.004637	0.007250	0.007117
	0.002710	0.002679	0.003004	0.006875	0.010938
	0.002715	0.002689	0.002695	0.002910	0.008712
	0.002437	0.002818	0.006121	0.008387	0.007674
	0.003086	0.003923	0.005196	0.008281	0.006250
	0.002310	0.003037	0.003385	0.008970	0.010351
	0.002640	0.004084	0.004217	0.009563	0.007632
	0.003370	0.003202	0.004602	0.008472	0.006167
	0.004130	0.002217	0.003297	0.006944	0.008251
	0.002439	void	0.002673	0.007837	0.005856
	(N = 10)	(N = 9)	(N = 10)	(N = 10)	(N = 10)
E5	0.001404	0.001264	0.001610	0.003035	0.001838
	0.002115	0.000439	0.001182	0.003015	0.001543
	0.001267	0.000697	0.002154	0.002938	0.001445
	0.001250	0.001427	0.001496	0.003318	0.001906
	0.001017	0.000856	0.001144	0.004292	0.001293
	0.001224	0.001741	0.000896	0.002846	0.001544
	0.001736	0.001223	0.001616	0.004111	0.001613
	0.001502	0.001281	0.001969	0.004124	0.001936
	0.001302	0.001442	0.001873	0.003528	0.001824
	0.002115	0.001774	0.001610	0.001829	0.001441
	(N = 10)	(N = 10)	(N = 10)	(N = 10)	(N = 10)

Table 12. Concentrations of Isoxanthopterin Isolated from Female Bodies, Calculated from Fluorimetric Readings of Individual Samples and Expressed as µg/mg Wet Weight of Sample, Continued

Day	Isoxanthopterin Concentration ar 4	ar 5	ar 6	ar 11	ar 12
E10	0.001102	void	0.001493	0.002295	0.001502
	0.001882	0.000706	0.001239	0.001419	0.001281
	0.000428	0.001282	0.000872	0.001597	0.001129
	0.001373	0.001175	0.001663	0.001735	0.001051
	0.001231	0.001557	0.001684	0.002106	0.001308
	0.001368	0.001184	0.001337	0.001162	0.001200
	0.001226	0.002023	0.002058	0.001798	0.001306
	0.001223	0.001335	0.002074	0.001539	0.001095
	0.001290	0.001282	0.001460	0.001307	0.001126
	0.001186	0.001404	0.001377	0.001466	0.001065
	(N = 10)	(N = 9)	(N = 10)	(N = 10)	(N = 10)
E15	0.000955	0.000856	0.001260	0.001076	0.001528
	0.000940	0.001083	0.001211	0.001531	0.001008
	0.000697	0.001901	0.000977	0.000983	0.001080
	0.000806	0.001214	0.001223	0.002539	0.000877
	0.001087	0.001374	0.001963	0.001616	0.001169
	0.001007	0.001168	0.000904	0.001160	0.001144
	0.000997	0.001540	0.000980	0.001411	0.001269
	0.000893	0.001439	0.001494	0.001389	0.001085
	0.001288	0.001585	0.001245	0.001108	0.000841
	0.000971	0.001056	0.000954	0.001574	0.000650
	(N = 10)	(N = 10)	(N = 10)	(N = 10)	(N = 10)